ESTROGEN RECEPTOR α REGULATED GENE EXPRESSION, RELATED ASSAYS AND THERAPEUTICS

FIELD OF THE INVENTION

The present disclosure relates to a plurality of genes modulated by estrogen or other agents, such as hormones or combinations of hormones, in various types of tissue. In particular, one embodiment of the disclosure relates to a plurality of genes which demonstrates certain patterns of expression differing qualitatively or quantitatively, with and without exposure to estrogen and/or other hormone compositions. The disclosure further relates to the methods of using these genes in identifying agents that exert at least some of the biological effects of estrogen and/or other agents, and to pharmaceuticals and related therapies. The disclosure further relates to the use of the plurality of genes in methods of monitoring, in gene chips and in kits.

BACKGROUND OF THE INVENTION

Estrogens exert biological effects in numerous organs throughout the body. The role of estrogens in reproductive biology, the prevention of postmenopausal hot flashes, and the prevention of postmenopausal osteoporosis are well established. Many observational studies have suggested estrogens also reduce the risk of development of cardiovascular disease(1), at least in part by estrogens reducing LDL cholesterol levels and elevating HDL cholesterol levels(2,3). More recently, estrogens

have been suggested to inhibit the development of colon cancer(4), inhibit the development of Alzheimer's disease(5), and inhibit development of cataracts (6). The multitude of estrogen responses matches the widespread distribution of estrogen receptors (ER) throughout numerous organs, with ERα expression highest in uterus, pituitary, kidney and adrenal gland and ERβ expression highest in ovary, uterus, bladder and lung(7). While various estrogens have been profiled for biological activity, little is known regarding the patterns of gene expression which are responsible for these diverse activities.

Thus, a need exists for the systemic analysis of the regulation by estrogen and/or other hormonal compositions of gene expression in various tissues and the identification of the plurality of differentially expressed genes. The identification of candidate agents that at least partially exert the same differential expression and development of pharmaceuticals and new treatment methods based on such agents is highly desirable. There also exists a need for methods of monitoring conditions and for diagnostic products, including gene chips and kits, which may be used in the above-described analyses.

The embodiments provided herein relate generally to a plurality of genes, particularly a plurality of genes that are modulated by estrogen and/or other hormonal compositions in various organs, such as the uterus, kidney and pituitary gland. Such differentially expressed genes are useful in screening assays to examine the effects of a candidate agent on the expression of genes that are responsive to estrogen. A candidate agent that induces, in a given tissue, a gene expression profile that exhibits

one or more similarities to the gene expression profile of estrogen and/or other hormonal compositions, can be identified for possible use in pharmaceuticals. The invention also relates to the identification of estrogen responsive genes that are known to be associated with the inhibition of certain conditions, such as shock, post-menopausal calcium deficiencies, cardiovascular diseases, and conditions where there is decreased renal blood flow, such as those caused by diuretics or congestive heart failure.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts a pattern analysis generated by a GeneChip microarray analysis. Specifically, WT or ERβKO ovariectomized mice were treated daily with vehicle or 20 μg/kg/day E2 for six weeks. Two hours following the final dose, the mice were euthanized and 13 tissues removed for RNA preparation. Two independent studies were performed, with total RNA pooled from two groups of three animals for each condition. Gene expression was quantified by GeneChip microarrays using murine U74 sub A arrays. Data were analyzed for patterns indicating either ERα or ERβ dependent regulation as shown. For ERα regulation, the defined search patterns (induction or repression) were for regulation by E2 in both wild type and ERβKO mice, in both sets of mice in both studies. For ERβ regulation, the defined search patterns were for regulation by E2 only in the wild type mice, with no change in basal expression in the ERβKO mice compared to the wild type mice. The number of genes in each tissue that matched the theoretical induction (↑) or repression (↓)patterns for ERα or ERβ are indicated.

Figures 2A-2C show a series of bar graphs showing gene expression levels of known genes regulated in the kidney in an ERα pattern. The expression levels (parts-per-million) are shown for the indicated genes in WT mice treated with vehicle (light blue bars), WT mice treated with E2 (dark blue bars), ERβKO mice treated with vehicle (light green bars), and ERβKO mice treated with E2 (dark green bars) using U74v2 subA, B, and C microarrays. Expression was measured in two independent sets of animals, with two groups of animals for each treatment in each study. A gene name abbreviation is shown above each graph, with the corresponding Unigene designation shown below. The genes are graphed in approximate order of regulation from largest induction (CYP7B1) to largest repression (BHMT).

Figure 3 shows histological sections of the kidney in in situ hybridization studies using antisense probes for CYP7B1, TF, STAT5A or GADD45G in ovariectomized mice treated with vehicle or 20 μg/kg/day E2 for six weeks. No signal was detected with the corresponding sense probes.

Figure 4 shows histological sections of the kidney in in situ hybridization using antisense probes for STAT5A or GAD45G in ovariectomized rats treated with vehicle or 20 μg/kg/day E2 for six weeks. No signal was detected with the corresponding sense probes.

Figure 5A-5B show a series of graphs showing expression levels for various genes. Ovariectomized WT mice were treated with vehicle or various doses of E2 for six weeks. (A) Kidney gene expression values (mean ± SEM) were determined by real-time PCR for each individual animal and normalized for GAPDH expression. The

mean expression level in vehicle-treated mice was defined as 1 for each gene. (B) Uterine wet weights (mg) and gene expression values (mean ± SEM).

Figures 6A-6D show a series of bar graphs depicting relative expression levels for various genes. Ovariectomized WT mice were treated with vehicle, 20 μg/kg/day E2, 5 mg/kg/day W-0292, W-0070 or propylpyrazole triol (PPT) for six weeks. Kidney gene expression values were determined by real-time PCR for each individual animal and normalized for GAPDH expression. The mean expression level in vehicle-treated mice was defined as 1 for each gene. *$p < 0.01$ for comparison to vehicle treated animals.

Figures 7A-7D show bar graphs depicting expression levels of intact and ΔAF1-ERα mRNA determined in uterus and kidney by using a real-time PCR assay specific for exon 3 of the mouse ERα or ERβ genes. Each graph utilizes a different scale. Expression levels were normalized for total RNA level to avoid GAPDH expression differences between kidney and uterus.

Figures 8A-8C show a series of relative expression levels for various genes in different types of mice. This figure also presents a model for AF1 or AF2 activation for each gene. Ovariectomized WT mice, ERαERβKO mice (expressing only ΔAF1-ERα) or ERαKO mice (expressing ΔAF1-ERα along with ERβ) were treated for 6 weeks with vehicle, 10 μg/kg/day E2, 10 μg/kg/day E2 + 5 mg/kg/day ICI182780, or 5 mg/kg/day tamoxifen. Kidney gene expression values were determined by real-time PCR for each individual animal and normalized for GAPDH expression. The mean expression level in vehicle-treated WT mice was defined as 1 for each gene. *$p < 0.01$ for comparison to vehicle treated animals. A model for the requirement of AF1 or AF2 for activation of

each gene is shown below each graph. The change in ER shape with tamoxifen (T) bound denotes the alternate helix 12 conformation induced by tamoxifen compared to E2. CA denotes coactivators.

SUMMARY OF EMBODIMENTS

One embodiment of the disclosure relates to a plurality of genes, each of whom is differentially expressed in tissue cells exposed to estrogen and/or other hormones or combination of hormones and tissue cells without said exposure, which plurality comprises a first group and a second group, wherein each gene in said first group is differentially expressed at a higher level in said tissue cells exposed to estrogen and/or a hormone or combinations of hormones than in said tissue cells without said exposure, wherein each gene in said second group is differentially expressed at a lower level in said tissue cells exposed to estrogen and/or a hormone or combinations of hormones than in said tissue cells without said exposure. Confirmation of such expression is confirmed by real-time PCR. Such cells preferably are from the kidney, pituitary or uterus. Exposure to estrogen and/or the other hormones is *in vivo* or *in vitro*. The higher level and lower levels are assessed using a predetermined statistical significance standard based on measurements of expression levels. The measurements can obtained using nucleotide arrays or nucleotide filters.

Another embodiment relates to a method for identifying an agent having the biological effect of estrogen and/or other hormones or combination of hormones, on gene expression in a given tissue, wherein said desired effect represents a first plurality of genes differentially expressed at various levels, which method comprises:

exposing, *in* vivo or *in vitro*, tissue cells to said agent;

measuring expression levels of a multiplicity of genes in said tissue cells exposed to said agent and tissue cells without said exposure, said multiplicity being greater than said first plurality;

determining, using a predetermined statistical significance standard, genes which are differentially expressed in said tissue cells exposed to said agent and said tissue cells without said exposure, said genes constitute a second plurality; and

comparing the expression levels of genes in said second plurality with the expression levels of genes in said first plurality,

wherein said agent is identified as having said desired effort if said first and second pluralities are the same and said expression levels in said first and second pluralities are substantially the same. The tissue preferably is kidney, uterus or pituitary tissue. Expression levels are confirmed by real-time PCR.

Another embodiment is directed to an agent identified by the above method.

Another embodiment is a pharmaceutical composition comprising this agent and a pharmaceutically acceptable excipient.

Another embodiment relates to a method for identifying an agent capable of maintaining vascular volume in septic shock, which method comprises:

exposing, *in vivo* or *in vitro*, kidney cells to the agent;

measuring expression levels of NTT73 and ABCC3 in said kidney cells exposed to the agent and kidney cells without the exposure;

comparing the expression levels of NTT73and ABCC3 with the expression levels of genes in the plurality if genes described above with regard to the kidney, wherein the induced genes are NTT73 and ABCC3,

wherein said agent is identified as capable of maintaining vascular volume in septic shock if said expression levels of NTT73 and ABCC3 are substantially the same as said expression levels of genes in such plurality.

Another embodiment relates to a method of identifying an agent capable of enhancing calcium uptake in post-menopausal women, which method comprises:

exposing, *in vivo* or *in vitro*, kidney cells to said agent;

measuring expression levels of CYP7B1 in said kidney cells exposed to said agent and kidney cells without said exposure;

comparing the expression levels of CYP7B1 with the expression levels of genes in the plurality of genes in the kidney is induced CYP7B1,

wherein said agent is identified as capable of enhancing calcium uptake in post-menopausal women if said expression levels of CYP7B1 are substantially the same as said expression levels of genes in such plurality.

Another embodiment relates to a method for identifying an agent for treating cardiovascular disorders, which method comprises:

exposing, *in vivo* or *in vitro*, kidney cells to said agent;

measuring expression levels of BHMT and SAHH in said kidney cells exposed to said agent and kidney cells without said exposure;

comparing the expression levels of BHMT and SAHH with the expression levels of genes in the plurality of genes, wherein in the kidney BHMT and SAHH are repressed,

wherein said agent is identified for treating cardiovascular disorders if said expression levels of BHMT and SAHH are substantially the same as said expression levels of genes in the such plurality.

Another embodiment relates to agents identified by any of the above methods and pharmaceutical agents comprising such agents and a pharmaceutically acceptable excipient.

Another embodiment relates to a solid substrate comprising any of the above described plurality of genes.

Another embodiment relates to a kit comprising any of the above plurality of genes.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The embodiments herein provide a plurality of genes modulated by estrogen and/or other hormonal compositions of interest in various types of tissue and the use of such a plurality of differentially expressed genes in screening for agents that exert at least some of the biological effects of estrogen and other hormonal compositions of

interest. Such identified agents can be used in pharmaceuticals and in related new therapeutic methods. The plurality of genes can be used in methods of monitoring.

Definitions:

In general, "**a gene**" is a region on the genome that is capable of being transcribed to an RNA that either has a regulatory function, a catalytic function and/or encodes a protein. A gene typically has introns and exons, which may organize to produce different RNA splice variants that encode alternative versions of a mature protein. "Gene" contemplates fragments of genes that may or may not represent a functional domain.

A "**plurality of genes**" as used herein refers to a group of identified or isolated genes whose levels of expression vary in different tissues, cells or under different conditions or biological states. The different conditions may be caused by exposure to certain agent(s) - whether exogenous or endogenous - which include hormones, receptor ligands, chemical compounds, etc. The expression of a plurality of genes demonstrates certain patterns. That is, each gene in the plurality is expressed differently in different conditions or with or without exposure to a certain endogenous or exogenous agents. The extent or level of differential expression of each gene may vary in the plurality and may be determined qualitatively and/or quantitatively according to this invention. A gene expression profile, as used herein, refers to a plurality of genes that are differentially expressed at different levels, which constitutes a "pattern" or a "profile." As used herein, the term "expression profile," "profile," "expression pattern," "pattern," "gene expression profile," and "gene expression

pattern" are used interchangeably.

An "**agent that exerts at least some of the biological effects of estrogen**," as used herein refers to any factor, agent, compound whether endogenous or exogenous in origin, which is capable of binding and interacting with estrogen receptors and thereby eliciting certain biological effects of extrogen. The skilled artisan would know that, for instance, one of the biological effects of estrogen is to promote the development of the female reproductive system. Other biological effects of estrogen are well documented and discussed, infra.

Gene expression profiles may be measured, according to this invention, by using nucleotide or microarrays. These arrays allow tens of thousands of genes to be surveyed at the same time.

"**Hormones or combinations of hormones**" include for instance, combinations of estrogens or other hormones that are known to exert biological effects of estrogen.

As used herein, the term "**microarray**" refers to nucleotide arrays that can be used to detect biomolecules, for instance to measure gene expression. "Array," "slide," and "chip" are used interchangeably in this disclosure. Various kinds of arrays are made in research and manufacturing facilities worldwide, some of which are available commercially. There are, for example, two main kinds of nucleotide arrays that differ in the manner in which the nucleic acid materials are placed onto the array substrate: spotted arrays and *in situ* synthesized arrays. One of the most widely used

oligonucleotide arrays is GeneChip™ made by Affymetrix, Inc. The oligonucleotide probes that are 20- or 25-base long are synthesized in silico on the array substrate. These arrays tend to achieve high densities (e.g., more than 40,000 genes per cm^2). The spotted arrays, on the other hand, tend to have lower densities, but the probes, typically partial cDNA molecules, usually are much longer than 20- or 25-mers. A representative type of spotted cDNA array is LifeArray made by Incyte Genomics. Pre-synthesized and amplified cDNA sequences are attached to the substrate of these kinds of arrays.

In one embodiment, the nucleotide is an array (i.e., a matrix) in which each position represents a discrete binding site for a product encoded by a gene (e.g., a protein or RNA), and in which binding sites are present for products of most or almost all of the genes in the organism's genome. In one embodiment, the "binding site" (hereinafter, "site") is a nucleic acid or nucleic acid analogue to which a particular cognate cDNA can specifically hybridize. The nucleic acid or analogue of the binding site can be, e.g., a synthetic oligomer, a full-length cDNA, a less-than full length cDNA, or a gene fragment.

Although the microarray may contain binding sites for products of all or almost all genes in the target organism's genome, such comprehensiveness is not necessarily required. Usually the microarray will have binding sites corresponding to at least about 50% of the genes in the genome, often at least about 75%, more often at least about 85%, even more often more than about 90%, and most often at least about 99%. Preferably, the microarray has binding sites for genes relevant to the action of the gene

expression modulating agent of interest or in a biological pathway of interest.

The nucleic acid or analogue are attached to a "**solid support**," which may be made from glass, plastic (e.g., polypropylene, nylon), polyacrylamide, nitrocellulose, or other materials. A preferred method for attaching the nucleic acids to a surface is by printing on glass plates, as is described generally by Schena et al., 1995, Quantitative monitoring of gene expression patterns with a complementary DNA microarray, Science 270:467-470. This method is especially useful for preparing microarrays of cDNA. See also DeRisi et al., 1996, Use of a cDNA microarray to analyze gene expression patterns in human cancer, Nature Genetics 14:457-460; Shalon et al., 1996, A DNA microarray system for analyzing complex DNA samples using two-color fluorescent probe hybridization, Genome Res. 6:639-645; and Schena et al., 1995, Parallel human genome analysis; microarray-based expression of 1000 genes, Proc. Natl. Acad. Sci. USA 93:10539-11286.

In a preferred embodiment, the microarray is a high-density oligonucleotide array, as described above. In a particularly preferred embodiment, the nucleotide arrays are the MG_U74 and MG_U74v2 arrays from Affymetrix.

"**Polymerase Chain Reaction**" or "**PCR**" is an amplification-based assay used to measure the copy number of the gene. In such assays, the corresponding nucleic acid sequences act as a template in an amplification reaction. In a quantitative amplification, the amount of amplification product will be proportional to the amount of template in the original sample. Comparison to appropriate controls provides a measure of the copy number of the gene, corresponding to the specific probe used,

according to the principle discussed above. Methods of "**real-time quantitative PCR**" using Taqman probes are well known in the art. Detailed protocols for real-time quantitative PCR are provided, for example, for RNA in: Gibson *et al.,* 1996, A novel method for real time quantitative RT-PCR. *Genome Res.* 10:995-1001; and for DNA in: Heid *et al.,* 1996, Real time quantitative PCR. *Genome Res.* 10:986-994.

A TaqMan-based assay can also be used to quantify polynucleotides. TaqMan based assays use a fluorogenic oligonucleotide probe that contains a 5' fluorescent dye and a 3' quenching agent. The probe hybridizes to a PCR product, but cannot itself be extended due to a blocking agent at the 3' end. When the PCR product is amplified in subsequent cycles, the 5' nuclease activity of the polymerase, for example, AmpliTaq, results in the cleavage of the TaqMan probe. This cleavage separates the 5' fluorescent dye and the 3' quenching agent, thereby resulting in an increase in fluorescence as a function of amplification (see, for example, http://www2.perkin-elmer.com).

Other suitable amplification methods include, but are not limited to, ligase chain reaction (LCR) (see, Wu and Wallace, 1989, *Genomics* 4: 560; Landegren *et al.*, 1988 *Science* 241: 1077; and Barringer *et al.*, 1990, *Gene* 89: 117), transcription amplification (Kwoh *et al.*, 1989, *Proc. Natl. Acad. Sci. USA* 86: 1173), self-sustained sequence replication (Guatelli *et al.*, 1990, *Proc. Nat. Acad. Sci. USA* 87: 1874), dot PCR, and linker adapter PCR, *etc.*

The "**level of mRNA**" in a biological sample refers to the amount of mRNA transcribed from a given gene that is present in a cell or a biological sample. One

aspect of the biological state of a biological sample (e.g. a cell or cell culture) usefully measured in the present invention is its transcriptional state. The transcriptional state of a biological sample includes the identities and abundances of the constituent RNA species, especially mRNAs, in the cell under a given set of conditions. Preferably, a substantial fraction of all constituent RNA species in the biological sample are measured, but at least a sufficient fraction is measured to characterize the action of an agent or gene modulator of interest. The level of mRNA may be quantified by methods described herein or may be simply detected, by visual detection by a human, with or without comparison to a level from a control sample or a level expected of a control sample.

A "**biological sample,**" as used herein refers to any sample taken from a biological subject, *in vivo* or *in situ*. A biological sample may be a sample of biological tissue, or cells or a biological fluid. Biological samples may be taken, according to this invention, from any kind of biological species, any types of tissues, and any types of cells, among other things. Cell samples may be isolated cells, primary cell cultures, or cultured cell lines according to this invention. Biological samples may be combined or pooled as needed in various embodimets. Preferred tissues are from the uterus, kidney, pituitary glands, breast, brain and adipose tissue.

"**Modulation of gene expression,**" as this term is used herein, refers to the induction or inhibition of expression of a gene. Such modulation may be assessed or measured by assays. Typically, modulation of gene expression may be caused by endogenous or exogenous factors or agents. The effect of a given compound can be

measured by any means known to those skilled in the art. For example, expression levels may be measured by PCR, Northern blotting, Primer Extension, Differential Display techniques, etc.

"**Induction of expression**" as used herein refers to any observable or measurable increase in the levels of expression of a particular gene, either qualitatively or quantitatively. The measurement of levels of expression may be carried out according to this invention using any techniques that are capable of measuring RNA transcripts in a biological sample. Examples of these techniques include, as discussed above, PCR, TaqMan, Primer Extension, Differential display and nucleotide arrays, among other things.

"**Repression of expression.**" "Repression" or "inhibition" of expression, are used interchangeably according to this disclosure. It refers to any observable or measurable decrease in the levels of expression of a particular gene, either qualitatively or quantitatively. The measurement of levels of expression may be carried out using any techniques that are capable of measuring RNA transcripts in a biological sample. The examples of these techniques include, as discussed above, PCR, TaqMan, Primer Extension, Differential Display, and nucleotide arrays, among other things."

A "**gene chip**" or "DNA chip" is described, for instance, in U.S. Patent Nos. 5,412,087, 5,445,934 and 5,744,305 and is useful for screening gene expression at the mRNA level. Gene chips are commercially available.

A "**kit**" is one or more of containers or packages, containing at least one "plurality of genes," as described above, on a solid support. Such kit also may contain various reagents or solutions, as well as instructions for use and labels.

A "**detectable label**" or a "**detectable moiety**" is a composition that when linked with a nucleic acid or a protein molecule of interest renders the latter detectable, via spectroscopic, photochemical, biochemical, immunochemical, or chemical means. For example, useful labels include radioactive isotopes, magnetic beads, metallic beads, colloidal particles, fluorescent dyes, electron-dense reagents, enzymes, biotin, digoxigenenin or haptens. A "labeled nucleic acid or oligonucleotide probe" is one that is bound, either covalently, through a linker or a chemical bond, or noncovalently through ionic, vander Waals, electrostatic, hydrophobic interactions, or hydrogen bonds, to a label such that the presence of the nucleic acid or probe may be detected by detecting the presence of the label bound to the nucleic acid or probe.

A "**nucleic acid probe**" is a nucleic acid capable of binding to a target nucleic acid or complementary sequence through one or more types of chemical bond, usually through complementary base pairing usually through hydrogen bond formation. As used herein, a probe may include natural (i.e., A, G, C, or T or modified bases (7-deazaguanosine, inosine, etc.). In addition, the bases in a probe may be joined by a linkage other than a phosphodiester bond, so long as it does not interfere with hybridization. It will be understood by one of skill in the art that probes may bind target sequences that lack complete complementarity with the probe sequence depending upon the stringency of the hybridization conditions. The probes are preferably directly

labeled with isotopes, for example, chromophores, luminphores, chromogens, or indirectly labeled with biotin to which a strepavidin complex may later bind. By assaying the presence or absence of the probe, one can detect the presence or absence of a target gene of interest.

"**In situ hybridization**" is a methodology for determining the presence of or the copy number of a gene in a sample, for example, fluorescence in situ hybridization (FISH) (see Angerer, 1987 *Meth. Enzymol* 152: 649). Generally, in situ hybridization comprises the following major steps: (1) fixation of tissue or biological structure to be analyzed; (2) prehybridization treatment of the biological structure to increase accessibility of target nucleic acid, and to reduce nonspecific binding; (3) hybridization of the mixture of nucleic acids to the nucleic acid in the biological structure or tissue; (4) post-hybridization washes to remove nucleic acid fragments not bound in the hybridization, and (5) detection of the hybridized nucleic acid fragments. The probes used in such applications are typically labeled, for example, with radioisotopes or fluorescent reporters. Preferred probes are sufficiently long, for example, from about 50, 100, or 200 nucleotides to about 1000 or more nucleotides, to enable specific hybridization with the target nucleic acid(s) under stringent conditions.

Hybridization protocols suitable for use with the methods of the invention are described, for example, in Albertson (1984) *EMBO J.* 3:1227-1234; Pinkel (1988) *Proc. Natl. Acad. Sci.* USA 85:9138-9142; *EPO* Pub. No. 430:402; *Methods in Molecular Biology,* Vol. 33: *In Situ* Hybridization Protocols, Choo, ed., Humana Press, Totowa, NJ (1994); *etc.*

"A predetermined statistical significance standard based on measurements of expression levels" is a confidence score based upon the assessment of four factors. Specifically, a score is assigned to each gene that reflects the confidence of the change. The score is based on four criteria: Fold Change, p-value (T-test), Present Calls, Frequency Value. For example, see the Table below:

TABLE I - Confidence criteria

Fold Change	Score
>2.0	5
>1.5	0
<1.5	-3

pValue	Score
<0.05	3
0.05 to 0.1	2
0.1 to 0.2	0
0.2 to 0.3	-1
0.3 to 0.5	-3

Present Calls	Score
2-4	3
1	1
0	0

Second Largest Frequency

>20	**3**
15 to 191	
10-14	**-1**
<10	**-3**

Outliers (Max Freq/2nd largest)

>2.5	**-3**

"**Data**" refers to information obtained that relates to the expression of genes in response to exposure to estrogen or an agent of unknown biological effect. The plurality of genes identified by the disclosed methods are examples of such information. The information is stored in electronic or paper formats. Electronic format can be selected from the group consisting of electronic mail, disk, compact disk (CD) digital versatile disk (DVD), memory card, memory chip, ROM or RAM, magnetic optical disk, tape, video, video clip, microfilm, internet, shared network, shared server and the like; wherein data is displayed, transmitted or analyzed via electronic transmission, video display, telecommunication, or by using any of the above stored formats; wherein data is compared and compiled at the site of sampling specimens or at a location where the data is transported following a process as described above.

"Genes modulated by estrogen." Genes regulated by estrogen and/or hormonal compositions and identified according to the disclosed methods, are listed in Tables II, III and IV. Relevant Unigene codes or Genbank accession numbers are provided.

Identification of Genes Modulated by Estrogen

A. Biological Sample and Assay

One embodiment disclosed herein relates to a plurality of genes, each of which is differentially expressed in kidney cells exposed to estrogen or a candidate agent and kidney cells without exposure to estrogen or a candidate agent, which plurality comprises a first group and a second group, wherein each gene in said first group is differentially expressed at a higher level in said kidney cells exposed to estrogen or a candidate agent than in said kidney cells without said exposure, wherein each gene in the second group is differentially expressed at a lower level in said kidney cells exposed to estrogen or candidate agent than in said kidney cells without said exposure.

A biological sample of kidney cells are obtained according to methods well known to the skilled artisan. One group of kidney cells are exposed to estrogen. Such estrogen may be 17ß estradiol. The kidney cells may be from one or more animals of the same species or from a culture of kidney cells or kidney tissue. Preferably, such cells are from a mammal, most preferably a mouse, rat or human. Such animal must produce little or no estrogen. For instance, an aromatase knockout animal cannot produce estrogen. Because the major source of circulating estrogen is the ovary, ovariectomy dramatically decreases circulating estrogen levels. Thus, in one embodiment, ovariectomized animals are used. By "exposure" is meant a type and quantity of either in vivo or in vitro administration that is applicable to the source of the kidney cells and known and acceptable to those of skill of the art. The total RNA from

such cells is prepared by methods known to the skilled artisan, e.g., by Trizol (Invitrogen) followed by subsequent repurification, e.g, via Rneasy columns (Qiagen). The total RNA is used to generate a labeled target according to methods and using detectable labels well know in the art, as described above in detail. For instance, the RNA may be labeled with biotin to form a cRNA target for use in an assay. See a complete description of preferred methods in the Affymetrix GeneChip® technical manual (Pages 700217 through 700223), which is herein incorporated by reference.

The assay, according to the invention, may be any assay suitable to detect gene expression. For instance, mRNA, cDNA or protein expression may be detected. Many different types of assays are known, examples of which are set forth above, including analyses by nucleotide arrays and nucleotide filters. The hybridization conditions (temperature, time, and concentrations) are adjusted according to procedures also well known in the art, as described above. In a preferred embodiment, the assay of the invention involves the use of a high density oligonucleotide array. For instance, in a preferred embodiment, cRNA labeled with biotin is hybridized to a murine MG_U74Av2 probe array (Affymetrix, Santa Clara, Ca.) for 16 hours at 45 degrees. Eleven biotin-labeled cRNAs at defined concentration are spiked into each hybridization and used to convert average difference values to frequencies expressed as parts per million.

Other solid supports and microarrays are known and commercially available to the skilled artisan, as described above.

B. Measurements and Statistical Analysis

The assay of the invention is used to identify genes modulated by estrogen. Such modulation may be induction of expression (a plurality of genes belonging to a "first group") or repression of such expression (a plurality of genes belonging to a "second group"). Gene expression induction is indicated by a higher level of expression, whereas repression is indicated by a lower level of expression, as assessed using a predetermined statistical significance standard based on measurements of expression levels.

Thus, the genes expressed or repressed in kidney cells with estrogen exposure are compared to the genes expressed or repressed in kidney cells that were not exposed to estrogen. Pairwise comparisons are made between each of the treatments. A pairwise comparison is the expression data for a given gene under a given treatment condition compared to the expression data for this gene under a second treatment condition. The fold change ratio is then calculated, the p-value based on Student's t-test, the number of present calls, and the expression level for each comparison. A confidence score "CS" is defined as CS(x)= FC(x) + PV(x) + PC(x) + EL(x) where FC, PV, PC and EL are scores assigned to the fold change, p-value, number of present calls, and the expression level, respectively. FC(x) is assigned 5 if the fold change ratio was greater then 1.95 and is assigned 0 if the ratio is between 1.95 and 1.5. PV(x) is assigned 3 if the p-value is less then 0.05 and is assigned 2 if the p-value was between 0.05 and 0.1. PC(x) is assigned 3 if at least 50% of the samples are called P by the Affymetrix algorithm and assigned 1 if only

25% of the samples are called P. EL(x) is assigned 3 if at least two samples have frequency value of 20 or greater and assigned 1 if two samples only have a frequency greater then 15. Penalty points are assigned if the fold change is less then 1.5, the p-value is greater then 0.2 or the frequency values were below 15 ppm. CS(x) ranged for –14 to 14 with qualifiers having a score of 14 considered the most significant changes. Genes with 11 or more points in any one pairwise comparison is considered to be significant. Real-time PCR and histology analyses are then performed to confirm the identity of the genes, essentially as described previously (9,10), which are herein incorporated by reference. The above described analysis can be used to identify candidate agents that are "estrogen-like" in that they have a differential expression profile which is in the most preferred embodiment substantially the same as estrogen's. For instance, in one embodiment, the expression levels for the genes upon exposure to the respective compounds is at least within 50% of each other.

C. Biological Samples from other Organs

The above described methods, assay and analysis can be applied to biological samples from any tissue, including the uterus, pituitary gland, liver, brain, colon, breast, adipose tissue, etc. In preferred embodiments, the biological samples are from the kidney, pituitary gland and the uterus.

D. The Plurality of Genes

Pursuant to the above described methods, the genes listed in Table II were identified as being differentially expressed upon exposure to estrogen. Genes in which

expression is induced by estrogen are considered to be genes of the "first group," whereas genes that are repressed by estrogen are considered to be in the "second group".

Specifically, the estrogen modulated genes in the kidney are Tissue Factor, CYP7B1, BCAT1, STAT5A, GADD45G, BHMT, SAHH, NTT73, ABCC3. Of these genes, estrogen induced expression in all but BHMT and SAHH, where it repressed expression.

Thus, one disclosed embodiment is a plurality of genes, wherein in the first group, where gene expression in kidney cells is induced by estrogen exposure, the plurality of genes comprise NTT73 and ABCC3. Another disclosed embodiment is a plurality of genes wherein the "first group" comprises CYP7B1 in kidney cells. In another embodiment, the plurality of genes of the "second group," where gene expression in kidney cells is repressed by estrogen exposure, comprises at least BHMT and SAHH.

Another disclosed embodiment is directed to a plurality of genes in kidney cells, wherein the first group comprises Tissue Factor, CYP7B1, BCAT1, STAT5A, and GADD45G, and wherein said second group comprises BHMT.

Another disclosed embodiment is directed to a plurality of the genes wherein the first group comprises CYP7B1, TF, SCYA28, Iga, Vk28, PHD 2, ELF 3, TIM1, STAT5A, COR1, BCAT1, ABCC3, TIM2, NAT6, RGS3, GNBP3, BCL7A, 17βDHH, FYVE ZFP, NTT73, AGPS, TRIM2, HBACH, CIS2, CYP27B1, and STAT5B, wherein

said second group comprises SAHH, ADH1A7, RARRES2, and BHMT. Another disclosed embodiment relates to a plurality of genes, wherein the first group comprises COR1 and GNBP3.

The estrogen modulated genes in the pituitary gland are STAT5B, GADD45G, Kallikrein-9, and FSHb, the expression of which is induced by estrogen for all but FSHb, which is repressed.

Thus one embodiment relates to a plurality of genes in the pituitary gland, wherein the first group comprises STAT5B and GADD45G.

Another embodiment relates to a plurality of genes, wherein the first group comprises STAT5B, GADD45G1 and Kallikreins genes in the pituitary.

Yet another embodiment relates to a plurality of genes, wherein the second group of genes in the pituitary gland comprise FSHb.

Pursuant to the above methods, the inventors discovered that the estrogen modulated genes in the uterus comprise SFRP4, Deiodinase (type II), Procollagen (type I, Alpha I), vimentin and IGFBP4, Scavenger receptor, AI121305, ALOX15, BCAT1, SiAMOX, C3, FOS, MAP2k1, CEBPb, EGR1 and CYP1A1. All of these genes are induced by estrogen in the uterus except for Scavenger receptor and CYP1A1, which are repressed.

Thus, one embodiment is directed to a plurality of genes in the uterus, wherein the first group comprises SFRP4, Deiodinase (type II), Procollagen (type I, Alpha I), vimentin and IGFBP4.

Another embodiment of the invention is directed to the plurality of genes, wherein the first group in the uterus comprises AI121305, ALOX15, BCAT1, SiAMOX, C3, FOX, MAP2k1, CEBPb and EGR1.

Another embodiment is directed to a plurality of genes wherein the first group in the uterus comprises SFRP4, Deiodinase (type II), Procollagen (type I, Alpha I), vimentin and IGFBP4, Scavenger receptor, AI121305, ALOX15, BCAT1, SiAMOX, C3, FOS, MAP2k1, CEBPb and EGR1.

In another embodiment, the plurality of genes in the second group in the uterus comprises CYP1A1.

In yet another embodiment, the plurality of genes in the second group in the uterus comprises Scavenger receptor.

Methods of identifying agents

Based upon the above described methods for determining differential expression of genes in various organs, another aspect of the invention relates to the identification of candidate agents that have the same or substantially the same biological effect of a known agent, such as estrogen or another hormonal combination of known biological effect. An "agent" could be any compound of unknown biological effect on genes in a given body site. Specifically, the invention relates to a method for identifying an agent having a desired effect on gene expression in an organ, wherein said desired effect represents a first plurality of genes differentially expressed at various levels, which method comprises:

exposing, *in vivo* or *in vitro*, organ cells to the agent;

measuring expression levels of a multiplicity of genes in the organ cells exposed to the agent and organ cells without the exposure, the multiplicity being greater than said first plurality;

determining, using a predetermined statistical significance standard, genes which are differentially expressed in the organ cells exposed to the agent and the organ cells without the exposure, the genes constitute a second plurality; and

comparing the expression levels of genes in the second plurality with the expression levels of genes in said first plurality,

wherein the agent is identified as having said desired effort if said first and second pluralities are the same and said expression levels in said first and second pluralities are substantially the same. The "organ cells" may be from any type of biological sample, as described above. In a preferred embodiment, such cells are from the kidney, pituitary gland or uterus. The "first plurality of genes" and "second plurality" of genes can be identified through a nucleotide array or filter, as described above. The comparing is performed using a suitable statistical technique with the assistance of known and commercially available programs, also as described above.

Another embodiment relates to an agent identified by the above method.

Yet another embodiment relates to a gene chip comprising any one or more of the above plurality of genes.

Pharmaceuticals and Methods of Treating

The identification of agents that induce or repress the expression of a gene associated with a given disorder or condition can lead to the development of pharmaceuticals that can be administered to a patient at therapeutically effective doses to prevent, treat, or control such disorder or condition.

Some conditions associated with estrogen regulation of gene expression in the kidney are known. For instance, in women, high estrogen levels preceding ovulation, during pregnancy, and resulting from estrogen administration commonly results in body water retention (23,24). Increased renal sodium reabsorption is a major mechanistic component for the elevated fluid retention (25). In rats, estrogen has been shown to increase thiazide-sensitive NaCl cotransporter expression levels(26), providing one possible molecular basis for estrogen effects on sodium retention.

Pursuant to the methods of the invention as disclosed above and as exemplified in greater detail in the Examples below, two additional estrogen regulated genes that influence sodium retention were identified. First, estrogen (E2) treatment increased mRNA levels for NTT73 (27), which is a sodium and chloride dependent transporter, known to regulate sodium retention. Second, E2 treatment also induced mRNA levels for ABCC3, a member of a family of genes which are known to modulate epithelial sodium channel activity (28). The physiological role of E2 regulation of these genes may lie in the large volume expansion required during pregnancy. The ED50 value for E2 activation of gene expression in the kidney was about 10-fold higher than that required for uterine weight increases (Figure 5), perhaps a mechanism to ensure

that normally estrogen actions only occur in the kidney when very high levels of estrogen are present, as during pregnancy.

Premenopausal women survive septic shock better than comparably aged males while postmenopausal women have a diminished survival advantage. Since volume loss is a major cause of morbidity in shock, it is expected that enhanced sodium and water retention due to elevated expression of NTT73(27) by E2 plays a role in this protective process. Thus, one disclosed embodiment relates to a method for identifying an agent capable of maintaining vascular volume in septic shock comprising exposing, *in vivo* or *in vitro*, kidney cells to an agent, measuring expression levels of NTT73 and ABCC3 in kidney cells exposed the agent and in kidney cells not exposed to the agent; comparing the expression levels of the NTT73 and ABCC3 with the expression levels of the genes kidney cells exposed to estrogen. The candidate agent identified by this process can be used in pharmaceuticals for purposes of maintaining vascular volume in the treatment of septic shock.

Another estrogen modulated gene in the kidney with biological significance is CYP27B1, the enzyme responsible for the rate limiting conversion of inactive 25-hydroxy vitamin D3 into active 1,25-dihidroxy vitamin D (29). This process is known to occur in the proximal tubules of the kidney and has been shown to be stimulated by estrogen treatment of birds(30). Urinary calcium excretion is increased in postmenopausal women, while estrogen treatment reduces urine calcium levels **(31, 32)**. The presence of vitamin D receptors within the proximal convoluted tubule and collecting duct tubules of the kidney suggests that E2 induction of CYP27B1 is the

basis of this beneficial effect.

Thus, the invention relates to a method of identifying agents that are capable of enhancing calcium uptake in postmenopausal women comprising exposing, *in vivo* or *in vitro*, kidney cells to an agent, measuring expression levels of CYP7B1 in kidney cells exposed the agent and in kidney cells not exposed to the agent; comparing the expression levels of the CYP7B1 with the expression levels of the genes in kidney cells exposed to estrogen. The agent identified by this process can be used in pharmaceuticals for purposes of enhancing calcium uptake in postmenopausal women.

It is known that estrogen treatment reduces expression of betaine:homocysteine methyltransferase (BHMT) and S-adenosylhomocysteine hydrolase (SAHH), two enzymes involved in the methionine / homocysteine cycle (34). Elevated plasma homocysteine levels are now recognized as an important risk factor for the development of cardiovascular disease **(35)**, and estrogen treatments reduced plasma homocysteine levels in postmenopausal women. Thus, the regulation of BHMT and SAHH provides a mechanistic link for this effect.

Thus, one embodiment disclosed herein relates to a method of identifying candidate agents for treating cardiovascular disorders comprising measuring expression of BHMT and SAHH in kidney cells exposed to an agent and in kidney cells with such exposure, comparing the expression levels of BHMT and SAHH with the expression levels of the genes in kidney cells exposed to estrogen. The agent identified by this process can be used in pharmaceuticals for purposes of treating

cardiovascular disorders.

Finally, E2 treatment induced expression of COR1 (chemokine orphan receptor 1, RDC1) an orphan G-protein coupled receptor (37), along with the guanylate nucleotide binding protein 3 (GNBP3) and the regulator of G-protein signaling 3 (RGS3), suggesting these proteins may form a functional unit. RDC1 is a receptor for the potent vasodilatory peptide adrenomedullin and calcitonin gene-related peptide, CGRP (38). Administration of CGRP to ovariectomized rats does not produce a decrease in kidney vascular resistance; however, in ovariectomized rats treated with E2 or in pregnant rats, injection of CGRP significantly decreases kidney vascular resistance (39). The observed increased expression of RDC1 in kidney provides a mechanism for the E2 induction of sensitivity to CGRP in the kidney, resulting in the large increase in renal flow seen during pregnancy (40).

Thus, one embodiment disclosed herein relates to a method of identifying candidate agents for treating conditions associated with reduced renal flow, such as caused by diuretics or congestive heart failure. Toxicity and therapeutic efficacy of such agents identified by the above methods can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, for example, for determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and can be expressed by the ratio, LD50/ED50. compounds that exhibit large therapeutic indices are preferred. While compounds that exhibit toxic side effects can be used, care should be taken to design

a delivery system that targets such compounds to the site of affected tissue to minimize potential damage to normal cells and thereby reduce side effects.

The data obtained from the cell culture assays and animal studies can be used to formulate a dosage range for use in humans. The dosage of such compounds likes preferably within a range of circulating concentrations that include ED50 with little or no toxicity. The dosage can vary within this range depending upon the dosage form employed and the route of administration.

The pharmaceuticals of the present invention can be formulated by standard techniques using one or more physiologically acceptable carriers or excipients and the biologically active agent. The agents and its physiologically acceptable salts and solvates can be formulated and administered orally, introraly, rectally, parenteraly, epicutaneously, topically, transdermally, subcutaneously, intramuscularly, intranasally, sublingually, intradurally, introcularly, intravenously , intraperioneally, or by inhalation.

With regard to oral administration, the pharmaceutical compositions can take the form of tablets or capsules prepared by conventional means with pharmaceutically acceptable excipient, such as binding agents etc. Tablets may be coated according to methods well known in the art. Liquid preparations can be in the form of solutions, suspensions and syrups or can be initially in dry form for constitution with water or other suitable vehicle. Other additives may include suspending agents, such as sorbitol syrup, cellulose derivatives or hydrogenated edible fats, emulsifying agents or non-aqueous solutions. Preparations for oral administration may also be formulated for a time or controlled release of the active ingredient using techniques well know in

the art of the invention.

Other formulations of the pharmaceuticals of the invention may be depot preparations for administration via implantation.

The pharmaceutical compositions of the present invention may be presented in a pack or dispenser device that contains one or more unit dosage forms containing the active ingredient. The pack can for example comprise metal or plastic foil, for example a blister pack. The pack or dispenser would contain instructions for administration.

Methods of Monitoring

The identification of the plurality of genes described above provides a powerful tool for assessing the progression of a state, condition or treatment. Specifically, a plurality of genes can be identified in a patient prior to an event, such as menopause, surgery, the onset of a therapeutic regime, or the completion of a therapeutic regime, to provide a base line result. This base-line can then be compared with the result obtained using identical methods either during or after such event. This information can be used for both diagnostic and prognostic purposes.

Kits

Another embodiment is directed to a kit containing a plurality of genes, preferably on a substrate. The kit also may comprise one or more containers or packages, along with reagents, solutions and possibly instructions for use.

* * *

All of the cited references are herein incorporated by reference. The invention is further described by the following Examples, which do not limit the invention in any manner.

Examples

Example 1: Introduction to Study and Animal-Related Procedures

Introduction

Estrogen receptors are expressed in numerous organs, although only a few organs are considered classical targets for estrogens. A systematic survey of estrogen regulation of approximately 10,000 genes in 13 tissues from wild type and ERβKO mice treated subcutaneously with vehicle or 17β-estradiol (E2) for six weeks was conducted. As expected, the uterus and pituitary had the greatest number of genes regulated by E2, while, surprisingly, the kidney had the third largest number of regulated genes. Some of these kidney regulations may provide mechanisms for known physiological effects of estrogens. For example, E2 induction of CYP27B1, the rate limiting enzyme in the synthesis of 1,25-dihydroxyvitamin D, may explain the ability of estrogens to decrease urinary calcium excretion in women. In situ hybridizations localized E2 regulation in the kidney to the juxtamedullary proximal and distal collecting tubule epithelial cells in both the mouse and rat. E2 regulations in the kidney were intact in the ERβKO mice, and the ERα selective agonist propyl pyrazole triol acted similarly as E2, together suggesting an ERα mediated mechanism. Finally,

the combination of the AF1-selective agonist tamoxifen plus mice expressing an AF1-deleted version of ERα (previously designated as ERα knockouts) allowed clear identification of genes dependent upon ERα AF1 activity and genes dependent upon ERα AF2 activity. Both AF1 and AF2 dependent genes were stimulated by E2 with the same ED_{50}, indicating that sensitivity of gene regulation in the kidney depends upon ER ligand binding and not on the subsequent ER activation mechanisms.

Animal-Related Procedure

Animals--Wildtype 129 strain female mice or Sprague-Dawley rats (bred at Wyeth or obtained from Taconic Farms) were placed on a casein-based diet at approximately 6 weeks of age. One week later, the animals were ovariectomized. Commencing the day after ovariectomy, each animal received a daily subcutaneous treatment with vehicle (50% DMSO, 50% phosphate buffered saline) or vehicle containing treatments for six weeks. Each group consisted of six or seven animals. Approximately 2 hours following the final treatment, the animals were euthanized with selected tissues frozen in liquid nitrogen for RNA analysis or on dry ice for histology.

Example 2: Preparation of Microarray

GeneChip--Total RNA was prepared separately from each individual organ by using Trizol (Invitrogen) followed by subsequent repurification on Rneasy columns (Qiagen). In general, two pools of RNA were created using equal amounts of RNA from three mice. For small organs such as pituitary, an equal amount of RNA from six animals was combined.

Target Preparation and Array Hybridization--Total RNA was used to generate biotin labeled cRNA target as described (8) which was hybridized to the murine MG_U74Av2 probe arrays (Affymetrix, Santa Clara, CA) for 16 h at 45°C. Eleven biotin-labeled cRNAs at defined concentration were spiked into each hybridization and used to convert average difference values to frequencies expressed as parts per million.

Example 3: Data Selection and Analysis

Pairwise comparisons were made between each of the treatments. We calculated the fold change ratio, the p-value based on Student's t-test, the number of present calls, and the expression level for each comparison. A confidence score (CS) was defined as CS(x)= FC(x) + PV(x) + PC(x) + EL(x) where FC, PV, PC and EL are scores assigned to the fold change, p-value, number of present calls, and the expression level, respectively. FC(x) was assigned 5 if the fold change ratio was greater then 1.95 and was assigned 0 if the ratio was between 1.95 and 1.5. PV(x) was assigned 3 if the p-value was less then 0.05 and was assigned 2 if the p-value was between 0.05 and 0.1. PC(x) was assigned 3 if at least 50% of the samples are called P by the Affymetrix algorithm and assigned 1 if only 25% of the samples are called P. EL(x) was assigned 3 if at least two samples had a frequency value of 20 or greater and assigned 1 if two samples only had a frequency greater then 15. Penalty points were assigned if the fold change was less then 1.5, the p-value was greater then 0.2 or the frequency values were below 15 ppm. CS(x) ranged for –14 to 14 with qualifiers having a score of 14 considered the most significant changes. Genes with 11 or more

points in any one pairwise comparison were considered to be significant and were included for further analysis. Real-time PCR on individual RNA samples and histology analyses were performed essentially as described previously (9, 10).

Example 4: Discussion of Results

To begin a systematic survey of estrogen receptor regulation of gene expression in the mouse, ovariectomized wild-type (WT) and ERβKO mice were treated by daily subcutaneous administration of either vehicle or 20 μg/kg/day 17β-estradiol (E2) for six weeks. RNA prepared from 13 organs was analyzed by microarray for estrogen regulation of gene expression. The resulting data set was queried for genes whose regulation was dependent on ERα or ERβ. For ERα regulation, the basal expression level was predicted to be the same in WT and ERβKO mice, with E2 induction or suppression occurring in both WT and ERβKO mice (Figure 1). For ERβ regulation, basal expression was predicted to remain constant, with E2 induction or suppression occurring in WT mice but not in ERβKO mice. ERα pattern regulations were found in well known estrogen target tissues such as the uterus (514 inductions, 19 repressions), pituitary (56 inductions, 30 repressions) and bone marrow (3 inductions, 3 repressions). In contrast, essentially no genes could be discerned that fit the predicted ERβ regulation pattern in any tissue.

Surprisingly, the kidney had a very large number of genes regulated at least 2-fold by E2 (26 inductions, 4 repressions; Figure 2). To further characterize E2 regulation of gene expression in the kidney, in situ hybridization was used to localize E2 induction of CYP7B1, TF, STAT5A and GADD45G. In each case, induction of gene

expression occurred in the juxtamedullary region of the kidney (Figure 3) primarily in the proximal and distal tubule epithelium (not shown). Estrogen regulation of STAT5A and GADD45G also occurred in rat kidney juxtamedullary region (Figure 4), demonstrating that the estrogen responsiveness of kidney is not limited to the mouse.

The ED_{50}s for E2 stimulation of CYP7B1, TF, STAT5A, and BCAT1 in the kidney were all very similar at about 3 µg/kg/day (Figure 5). Although this is approximately 10-fold greater than the ED_{50} dose of E2 required for uterine weight increases, the ED_{50} for gene induction in the uterus can vary by 20-fold, from 0.2 ug/kg/day E2 for BCAT1 induction to 2.7 ug/kg/day for c-fos (Figure 5). The E2 induction of gene expression in the kidney at the same dose as induction of well characterized genes such as c-fos in the uterus suggests that regulation of kidney gene expression occurs at physiological levels of E2.

Confirmation of the role of ERα in the induction of kidney gene expression was obtained with 4-propyl-1,3,5-Tris(4-hydroxy-phenyl) pyrazole (PPT) a compound which exclusively activates ERα but not ERβ (11). Treatment with PPT induced expression of CYP7B1, TF, STAT5A and BCAT1 to a similar extent as did treatment with E2 (Figure 6). Further, two ERβ selective agonists (W-0292 and W-0070, both approximately 75-fold selective for ERβ compared to ERα by in vitro binding assays; data not shown) failed to stimulate expression of any of these four genes (Figure 6). Finally, in agreement with previous results, ERα mRNA was detectable within the mouse kidney (Figure 7). The regulation of these genes in WT and ERβKO mice, the similar E2 ED_{50} for each gene, the activity of a selective ERα agonist, the inactivity of

selective ERβ agonists, and the expression of ERα within the kidney together suggest a single, ERα mediated pathway for regulation of these genes.

It has been recognized that a commonly utilized strain of ERαKO mice (12) in fact expresses an ERα protein lacking only AF1, due to alternative splicings of the exon containing the targeted knockout mutation(13, 14). The resulting truncated ERα proteins, referred to here as ΔAF1-ERα, have the ability to stimulate expression of a synthetic estrogen response element driven promoter (14). As found previously for ERαKO mice, the level of this misspliced transcript in the uterus of ERαERβKO mice was lower than the level of full length message in WT mice (Figure 7). Again as expected, the amount of intact ERα mRNA was much lower in the whole kidney than in uterus from WT mice. However, the level of ΔAF1-ERα mRNA was actually greater in the ERαERβKO kidney than was intact ERα mRNA in WT kidney. No ERβ mRNA could be detected in either uterus or kidney from the ERαERβKO mice.

The presence of ΔAF1-ERα at significant levels in the kidney allows determination of the relative contribution of AF1 and AF2 to E2 regulation of individual genes. To determine whether AF1 or AF2 regions of ERα were required for induction of CYP7B1, TF or BCAT1 in the kidney, WT, ERαERβKO or ERαKO mice were treated with E2 or the AF1 selective agonist tamoxifen (15). Expression of CYP7B1 was induced by E2 but not by tamoxifen in WT mice (Figure 8), suggesting that induction of CYP7B1 occurred through AF2. Consistent with this hypothesis, E2 also increased CYP7B1 expression in mice expressing ΔAF1-ERα (either ERαERβKO or ERαKO mice). This E2 induction was blocked by an excess of ICI-182780, confirming the

regulation occurred through ER. Together, these two lines of evidence suggest that induction of CYP7B1 is an AF2 dependent process. In contrast, TF expression was induced by both E2 and tamoxifen in WT mice. Neither compound could induce TF expression in ERαERβKO mice (which express only ΔAF1-ERα). This suggests that induction of TF occurs through an AF1 mediated pathway. Finally, BCAT1 was also induced by both E2 and tamoxifen in WT mice. However, in ERαERβKO mice, E2 stimulated BCAT1 expression but tamoxifen did not. These results suggest that BCAT1 expression can be stimulated through either AF1 or AF2 mechanisms. In WT mice, tamoxifen stimulates expression through AF1 only. Since ΔAF1-ERα lacks the AF1 region necessary for tamoxifen activity, tamoxifen cannot stimulate BCAT1 expression in ERαERβKO mice. In contrast, E2, which can stimulate BCAT1 expression through either AF1 or AF2, can still stimulate expression in the ΔAF1-ERα expressing mice.

Estrogen receptors α or β are found in almost all organs of the body, yet relatively few tissues are considered targets for estrogen action. To begin to develop a more complete understanding of estrogen biology, estrogen responsive genes in 13 tissues from WT and ERβKO mice were characterized. In general, many tissues showed patterns of E2 regulation consistent with an ERα mechanism, including such known target organs as uterus, pituitary, and bone. Surprisingly, no E2 regulations were found that fit the expected pattern for ERβ regulations. This was true even in organs expressing moderately high levels of ERβ such as the bladder and lung (7). At least three mechanisms could explain the lack of detection of expected ERβ responses. First, it has been proposed that a major function of ERβ is to modulate the

activity of ERα(16). For example, expression of the Ki-67 protein was constitutively elevated in uterus of ERβKO mice, i.e. in the ERβKO mice its expression was always equivalent to the E2 stimulated levels in WT animals (17). The survey criteria used here would not detect this pattern. Further analysis of these data has revealed many genes in multiple tissues which also have this "nonclassical" pattern of regulation whereby expression is constitutively elevated in both vehicle and E2 treated ERβKO mice (data not shown). Second, analysis of whole organs may easily miss regulations occurring in only selected cell subtypes within an organ. For example, initial analysis of kidney did not identify GADD45G as being regulated by E2, because GADD45G expression is regulated only in tubule epithelial cells. The unregulated expression of GADD45G throughout most of the kidney sufficiently diminished the fold induction so as to be less than 2-fold in whole organ samples. The combination of laser capture microdissection with microarray technology (18) should allow detection of ERβ regulated genes with a classical pattern of regulation.

This global survey demonstrates that, unexpectedly, the kidney had a very large number of regulated genes. Both genetic approaches (Figure 2) and pharmacological approaches (Figure 6) demonstrated that E2 regulation in the kidney was mediated through ERα. Expression of CYP7B1, TF, STAT5A, and even genes such as GADD45G which are expressed throughout the kidney showed regulation only in tubule epithelium (Figure 3). Additionally, KIM-1, the rat counterpart of mouse TIM1 and TIM2 (20) is also expressed in proximal tubule epithelial cells (21). Finally, ^{3}H-E2 binding localizes to proximal tubule cells following administration to rats(22).

Together, these results suggest that ERα directly regulates gene expression in tubule epithelial cells.

Although the observed regulations in the kidney were mediated by ERα, the mechanism of activation of gene expression by ERα was gene specific. Thus studies using tamoxifen, which activates ERα through AF1, along with studies using ΔAF1-ERαKO mice (previously designated as ERKO mice) together indicate that E2 induction of CYP7B1 expression occurred predominantly through an AF1-dependent mechanism, E2 induction of TF expression occurred predominantly through an AF2-dependent mechanism, and E2 induction of BCAT1 expression occurred through both AF1 and AF2 mechanisms (Figure 8). The ED_{50} values for E2 stimulation of these three genes were all very similar (Figure 5). Thus, whether a gene is induced through either AF1 or AF2 mechanism does not influence the sensitivity of the gene in the kidney to plasma estrogen levels. Rather, the data indicate that the binding of E2 to ERα would be the rate limiting step in induction of gene expression in the kidney. The maximal fold regulation varied between genes and may depend upon whether and AF1 or AF2 dependent pathway is utilized.

Analysis of 10 kb of upstream putative promoter sequences of E2 induced genes identified good matches to the consensus estrogen response element (ERE) in only a few genes, although ERE half-sites could be identified in most promoters. Many of these genes may be activated through nonclassical ERα mechanisms such as the combination of an ERE half-site with Sp1 binding sites (41). It is unlikely that a nonclassical ERα / AP1 stimulatory mechanism is responsible for these regulations,

since ICI182780 functions as a partial agonist in this mechanism (42) while ICI182780 was a complete antagonist for E2 regulation of gene expression in the kidney (Figure 8). Additionally, E2 induced expression of the transcription factors PHD2, ELF3, STAT5A and STAT5B. It is possible that E2 induction of these transcription factors resulted in the subsequent increase in expression of the remaining genes. For example, CIS2 is a known target for induction by STAT transcription factors (43), suggesting that the E2 induction of CIS2 is mediated indirectly through the E2 induction of STAT5A and STAT5B.

TABLE II – Genes Regulated By Estrogen in Kidney, Uterus and Pituitary Gland

Kidney	Unigene Code	Full name	Why
Tissue Factor	Mm.3742	Coagulation factor III	Mechanism is ERα AF1 dependent
CYP7B1	Mm.6216	Cytochrome P450, 40 (25-hydroxyvitamin D3 1 alpha-hydroxylase)	Mechanism is ERα AF2 dependent
BCAT1	Mm.4606	Branched chain aminotransferase 1, cytosolic	Mechanism is ERα AF1 + AF2 dependent
STAT5A	Mm.4697	Signal transducer and activator of transcription 5A	Regulated in multiple species (mouse and rat)
GADD45G	Mm.9653	Growth arrest and DNA-damage-inducible 45 gamma	Regulated in multiple species (mouse and rat)
BHMT	Mm.21983	Betaine-homocysteine methyltransferase	A repression by estrogens
SAHH	Mm.2573	S-adenosylhomocysteine hydrolase	
NTT73	Mm.4327	SODIUM- AND CHLORIDE-DEPENDENT TRANSPORTER NTT73	
ABCC3	Mm.23942	ATP-binding cassette, sub-family C (CFTR/MRP), member 3	

TABLE II – Genes Regulated By Estrogen in Kidney, Uterus and Pituitary Gland

Uterus	Unigene Code	Full name	Why
SFRP4	Mm.42095	Secreted frizzled-related sequence protein 4	Induced by estrogens in mouse uterus and human endometrium
Deiodinase, type II	Mm.21389	Deiodinase, iodothyronine, type II	Induced by estrogens in mouse uterus and human endometrium
Procollagen, type I, alpha 1	Mm.22621	Procollagen, type I, alpha 1	Induced by estrogens in mouse uterus and human endometrium
vimentin	Mm.7	Vimentin	Induced by estrogens in mouse uterus and human endometrium
IGFBP4	Mm.22248	Insulin-like growth factor binding protein 4	Induced by estrogens in mouse uterus and human endometrium
Scavenger receptor	Mm.1227	Macrophage scavenger receptor 1	Repressed by estrogens in mouse uterus and human endometrium
AI121305	Mm.29959	RIKEN cDNA 1600029D21	a set of genes induced by estrogens with a range of ED50 values
ALOX15	Mm.4584	Arachidonate 15-lipoxygenase	a set of genes induced by estrogens with a range of ED50 values
BCAT1	Mm.4606	Branched chain aminotransferase 1, cytosolic	a set of genes induced by estrogens with a range of ED50 values
SiAMOX	Mm.7190	Amiloride binding protein 1 (amine oxidase, copper-containing)	a set of genes induced by estrogens with a range of ED50 values
C3	Mm.19131	Complement component 3	a set of genes induced by estrogens with a range of ED50 values
FOS	Mm.5043	FBJ osteosarcoma oncogene	a set of genes induced by estrogens with a range of ED50 values
MAP2k1	Mm.1059	Mitogen activated protein kinase kinase 1	a set of genes induced by estrogens with a range of ED50 values
CEBPb	Mm.4863	CCAAT/enhancer binding protein (C/EBP), beta	a set of genes induced by estrogens with a range of ED50 values
EGR1	Mm.181959	Early growth response 1	a set of genes induced by estrogens with a range of ED50 values
CYP1A1	Mm.14089	Cytochrome P450, 1a1, aromatic compound inducible	Repressed by estrogens

TABLE II – Genes Regulated By Estrogen in Kidney, Uterus and Pituitary Gland

Pituitary	Unigene Code	Full name	Why
STAT5B	Mm.34064	Signal transducer and activator of transcription 5B	Induced by estrogens
GADD45G	Mm.9653	Growth arrest and DNA-damage-inducible 45 gamma	Induced by estrogens
Kallikrein-9	Mm.200410	Kallikrein 9	Induced by 17b-estradiol, not by Premarin
FSHb	Mm.46711	Follicle stimulating hormone beta	Repressed by estrogens

TABLE III - Genes Regulated By Estrogen in the Uterus

Mousedata. Qualifier	Pub_Name	Gene Name	Tissue	Mean WT E2 Fold Change
94120_s_at	SPRR2F	small proline-rich protein 2F	Uterus	38.71
97413_at	UNK_AI121305	ESTs, Weakly similar to AF189262_1 GABA-A receptor epsilon-like subunit [R.norvegicus]	Uterus	31.68
101130_at	COLA2	procollagen, type I, alpha 2	Uterus	29.57
103526_at	PDI2	peptidyl arginine deiminase, type II	Uterus	23.01
99059_at	ELF3	E74-like factor 3	Uterus	22.03
95343_at	PDI1	peptidyl arginine deiminase, type I	Uterus	21.72
101115_at	LTF	lactotransferrin	Uterus	19.01
93481_at	UNK_AI846720	Cluster Incl AI846720:UI-M-AN1-afi-h-09-0-UI.s1 Mus musculus cDNA, 3' end /clone=UI-M-AN1-afi-h-09-0-UI /clone_end=3' /gb=AI846720 /gi=5490626 /ug=Mm.7124 /len=161 /STRA=for	Uterus	17.96
93097_at	ARG1	arginase 1, liver	Uterus	17.45
104249_g_at	UNK_AW227650	ESTs, Highly similar to TRANSLOCON-ASSOCIATED PROTEIN, GAMMA SUBUNIT [Rattus norvegicus]	Uterus	16.87
93797_g_at	LDH1	lactate dehydrogenase 1, A chain	Uterus	16.5
101761_f_at	SPRR2C	small proline-rich protein 2C	Uterus	16.48
102805_at	CEACAM1	CEA-related cell adhesion molecule 1	Uterus	16
98064_at	GLYCAM1	glycosylation dependent cell adhesion molecule 1	Uterus	15.46
AFFX-GapdhMur/M32599_5_at	GAPDH5_Mm_AFFX	Glyceraldehyde-3-phospate dehydrogenase 5' control sequence (M. musculus) [AFFX]	Uterus	15.2
AFFX-GapdhMur/M32599_5_at	GAPDH5_Mm_AFFX	Glyceraldehyde-3-phospate dehydrogenase 5' control sequence (M. musculus) [AFFX]	Uterus	15.2
AFFX-GapdhMur/M32599_5_at	GAPDH5_Mm_AFFX	Glyceraldehyde-3-phospate dehydrogenase 5' control sequence (M. musculus) [AFFX]	Uterus	15.2
96605_at	UNK_AI787183	ESTs, Weakly similar to AF115426_1 LR8 [M.musculus]	Uterus	14.98

TABLE III - Genes Regulated By Estrogen in the Uterus

Mousedata. Qualifier	Pub_Name	Gene Name	Tissue	Mean WT E2 Fold Change
102806_g_at	CEACAM1	CEA-related cell adhesion molecule 1	Uterus	14.48
93860_i_at	UNK_M17327	Mouse endogenous murine leukemia virus modified polytropic provirus DNA, complete cds	Uterus	13.83
101707_at	ALDH1A7	alcohol dehydrogenase family 1, subfamily A7	Uterus	13.63
104486_at	UNK_AI850558	ESTs, Highly similar to ALPHA-2-MACROGLOBULIN PRECURSOR [Homo sapiens]	Uterus	13.29
98423_at	GJB2	gap junction membrane channel protein beta 2	Uterus	12.6
104182_at	HGFAC	hepatocyte growth factor activator	Uterus	12
94789_r_at	TUBB5	tubulin, beta 5	Uterus	11.55
98822_at	ISG15	interferon-stimulated protein (15 kDa)	Uterus	11.5
97826_at	UNK_AI465965	ESTs, Weakly similar to IgG Fc binding protein [M.musculus]	Uterus	10.95
100026_at	BCAT1	branched chain aminotransferase 1, cytosolic	Uterus	10.35
102316_at	CAPN5	calpain 5	Uterus	10.01
98092_at	D5WSU111E	DNA segment, Chr 5, Wayne State University 111, expressed	Uterus	9.77
102918_at	MUC1	mucin 1, transmembrane	Uterus	9.74
97173_f_at	H2-K2	histocompatibility 2, K region locus 2	Uterus	9.71
93497_at	C3	complement component 3	Uterus	9.63
93517_at	COL6A2	procollagen, type VI, alpha 2	Uterus	9.57
92796_at	AKP2	alkaline phosphatase 2, liver	Uterus	9.36
103824_at	WFS1	Wolfram syndrome 1 homolog (human)	Uterus	9.15
99378_f_at	UNK_M18837	Mouse MHC class I Q4 beta-2-microglobulin (Qb-1) gene, complete cds	Uterus	8.9
99561_f_at	CLDN7	claudin 7	Uterus	8.84
94305_at	COLA1	procollagen, type I, alpha 1	Uterus	8.78
92223_at	C1QC	complement component 1, q subcomponent, c polypeptide	Uterus	8.63
100134_at	ENG	endoglin	Uterus	8.53
92550_at	KRT1-19	keratin complex 1, acidic, gene 19	Uterus	8.53
103905_at	UNK_AI314958	ESTs, Highly similar to CARBONIC ANHYDRASE VI [Ovis aries]	Uterus	8.44
93285_at	UNK_AI845584	ESTs, Highly similar to DUS6_RAT DUAL SPECIFICITY PROTEIN PHOSPHATASE 6 [R.norvegicus]	Uterus	8.39
92777_at	CYR61	cysteine rich protein 61	Uterus	8.3

TABLE III - Genes Regulated By Estrogen in the Uterus

Mousedata. Qualifier	Pub_Name	Gene Name	Tissue	Mean WT E2 Fold Change
97819_at	GSTTL-PENDING	glutathione S-transferase like	Uterus	8.12
93479_at	UNK_AW122413	Cluster Incl AW122413:UI-M-BH2.2-aow-f-03-0-UI.s1 Mus musculus cDNA, 3' end /clone=UI-M-BH2.2-aow-f-03-0-UI /clone_end=3' /gb=AW122413 /gi=6097916 /ug=Mm.7113 /len=470 /STRA=rev	Uterus	7.99
104099_at	PGLYRP	peptidoglycan recognition protein	Uterus	7.98
97507_at	PPICAP	peptidylprolyl isomerase C-associated protein	Uterus	7.86
94876_f_at	UNK_AI849207	ESTs, Weakly similar to AF218940_1 formin-2 [M.musculus]	Uterus	7.77
96911_at	GNB2	guanine nucleotide binding protein, beta 2	Uterus	7.56
92608_at	CSRP	cysteine rich protein	Uterus	7.56
94269_at	RABAC1	Rab acceptor 1 (prenylated)	Uterus	7.46
93861_f_at	UNK_M17327	Mouse endogenous murine leukemia virus modified polytropic provirus DNA, complete cds	Uterus	7.39
101110_at	COL6A3	procollagen, type VI, alpha 3	Uterus	7.37
93974_at	33 POLYPEPTIDE□ [R.NORVEGICUS]	ESTs, Highly similar to G33_RAT GENE 33 POLYPEPTIDE□ [R.norvegicus]	Uterus	7.34
98758_at	ALOX15	arachidonate 15-lipoxygenase	Uterus	7.33
99379_f_at	UNK_M27034	Mouse MHC class I D-region cell surface antigen (D2d) gene, complete cds	Uterus	7.23
93078_at	LY6	lymphocyte antigen 6 complex	Uterus	7.17
93290_at	PNP	purine-nucleoside phosphorylase	Uterus	7.12
101979_at	GADD45G	growth arrest and DNA-damage-inducible 45 gamma	Uterus	7.06
99452_at	LISCH7-PENDING	liver-specific bHLH-Zip transcription factor	Uterus	7
94274_at	PFDN5	prefoldin 5	Uterus	6.97
92880_at	MFGE8	milk fat globule-EGF factor 8 protein	Uterus	6.95
101294_g_at	G6PD2	glucose-6-phosphate dehydrogenase 2	Uterus	6.95
92759_at	LAMB3	laminin, beta 3	Uterus	6.88
92585_at	MAP2K1	mitogen activated protein kinase kinase 1	Uterus	6.86
95232_at	HNRPL	heterogeneous nuclear ribonucleoprotein L	Uterus	6.79
104410_at	MIDN-PENDING	midnolin	Uterus	6.75
96075_at	WDR1	WD repeat domain 1	Uterus	6.71
95631_at	PPP4C	protein phosphatase 4, catalytic subunit	Uterus	6.68

TABLE III - Genes Regulated By Estrogen in the Uterus

Mousedata. Qualifier	Pub_Name	Gene Name	Tissue	Mean WT E2 Fold Change
100412_g_at	AEBP1	AE-binding protein 1	Uterus	6.67
96634_at	UNK_AI850090	ESTs, Weakly similar to cDNA EST EMBL:C07816 comes from this gene [C.elegans]	Uterus	6.65
97282_at	MELA	melanoma antigen, 80 kDa	Uterus	6.6
98511_at	RALY	hnRNP-associated with lethal yellow	Uterus	6.55
94199_at	KAP	kidney androgen regulated protein	Uterus	6.45
93818_g_at	RNP24-PENDING	coated vesicle membrane protein	Uterus	6.32
98331_at	COL3A1	procollagen, type III, alpha 1	Uterus	6.29
92642_at	CAR2	carbonic anhydrase 2	Uterus	6.28
103278_at	PDI4	peptidyl arginine deiminase, type IV	Uterus	6.23
101542_f_at	DDX3	DEAD (aspartate-glutamate-alanine-aspartate) box polypeptide 3	Uterus	6.23
93793_at	LASP1	LIM and SH3 protein 1	Uterus	6.17
94817_at	SERPINH1	serine (or cysteine) proteinase inhibitor, clade H (heat shock protein 47), member 1	Uterus	6.06
99569_at	KRT2-18	keratin complex 2, basic, gene 18	Uterus	6.04
95705_s_at	ACTX	melanoma X-actin	Uterus	5.94
92368_at	RAMP3	receptor (calcitonin) activity modifying protein 3	Uterus	5.93
102292_at	GADD45A	growth arrest and DNA-damage-inducible 45 alpha	Uterus	5.93
94384_at	IER3	immediate early response 3	Uterus	5.84
103438_at	DIO2	deiodinase, iodothyronine, type II	Uterus	5.83
97882_at	SEC61A	SEC61, alpha subunit (S. cerevisiae)	Uterus	5.81
93574_at	PEDF	pigment epithelium-derived factor	Uterus	5.81
99622_at	KLF4	Kruppel-like factor 4 (gut)	Uterus	5.74
100981_at	IFIT1	interferon-induced protein with tetratricopeptide repeats 1	Uterus	5.74
99645_at	UNK_AW048484	Cluster Incl AW048484:UI-M-BH1-alj-d-10-0-UI.s1 Mus musculus cDNA, 3' end /clone=UI-M-BH1-alj-d-10-0-UI /clone_end=3' /gb=AW048484 /gi=5909018 /ug=Mm.43640 /len=458 /STRA=for	Uterus	5.67
95444_at	UNK_AW122274	ESTs, Weakly similar to CG1534 gene product [D.melanogaster]	Uterus	5.66
99931_at	LAMA5	laminin, alpha 5	Uterus	5.66
100130_at	JUN	Jun oncogene	Uterus	5.64

TABLE III - Genes Regulated By Estrogen in the Uterus

Mousedata. Qualifier	Pub_Name	Gene Name	Tissue	Mean WT E2 Fold Change
100618_f_at	SLC25A5	solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 5	Uterus	5.64
101929_at	UNK_AI836322	Cluster Incl AI836322:UI-M-AQ0-aag-a-02-0-UI.s2 Mus musculus cDNA, 3' end /clone=UI-M-AQ0-aag-a-02-0-UI /clone_end=3' /gb=AI836322 /gi=5470530 /ug=Mm.939 /len=211 /STRA=for	Uterus	5.63
100609_at	UNK_AF049850	Cluster Incl AF049850:Mus musculus major histocompatibility locus class III region- complement C4 (C4) and cytochrome P450 hydroxylase A (CYP21OH-A) genes, complete cds; slp pseudogene, complete sequence; NG6, SKI, and complement factor B (Bf) genes, comp	Uterus	5.58
95637_at	UNK_AI838592	ESTs, Moderately similar to ENDOTHELIAL ACTIN-BINDING PROTEIN [Homo sapiens]	Uterus	5.46
101367_at	DCTN1	dynactin 1	Uterus	5.42
101681_f_at	H2-BL	histocompatibility 2, blastocyst	Uterus	5.24
100557_g_at	UNK_AW121930	ESTs, Highly similar to EUKARYOTIC INITIATION FACTOR 4B [Homo sapiens]	Uterus	5.21
93985_at	UNK_AW120868	ESTs, Highly similar to hypothetical protein [H.sapiens]	Uterus	5.18
92851_at	CP	ceruloplasmin	Uterus	5.14
99109_at	IER2	immediate early response 2	Uterus	5.13
99632_at	MAD2L1	MAD2 (mitotic arrest deficient, homolog)-like 1 (yeast)	Uterus	5.13
94307_at	FBLN1	fibulin 1	Uterus	5.1
92232_at	CISH3	cytokine inducible SH2-containing protein 3	Uterus	5.09
92611_at	GPIAP-PENDING	GPI-anchored membrane protein 1	Uterus	5.07
104333_at	D17H6S56E-5	DNA segment, Chr 17, human D6S56E 5	Uterus	5.07
101016_at	ARF1	ADP-ribosylation factor 1	Uterus	5.06
103460_at	UNK_AI849939	ESTs, Moderately similar to unnamed protein product [H.sapiens]	Uterus	5.05
94309_g_at	FBLN1	fibulin 1	Uterus	4.95
99927_at	CFI	complement component factor i	Uterus	4.94
96278_at	UNK_AI846553	ESTs, Weakly similar to DIA1_MOUSE DIAPHANOUS PROTEIN HOMOLOG 1 [M.musculus]	Uterus	4.84

TABLE III - Genes Regulated By Estrogen in the Uterus

Mousedata. Qualifier	Pub_Name	Gene Name	Tissue	Mean WT E2 Fold Change
103345_at	UNK_AW046708	ESTs, Highly similar to SPECTRIN ALPHA CHAIN, NON-ERYTHROID [Rattus norvegicus]	Uterus	4.83
95794_f_at	SPRR2I	small proline-rich protein 2I	Uterus	4.83
101908_s_at	CEACAM2	CEA-related cell adhesion molecule 2	Uterus	4.8
104144_at	GTPBP2	GTP binding protein 2	Uterus	4.8
102362_i_at	JUNB	Jun-B oncogene	Uterus	4.79
AFFX-GapdhMur/M32599_M_at	GAPDHM_Mm_AFFX	Glyceraldehyde-3-phospate dehydrogenase middle control sequence (M. musculus) [AFFX]	Uterus	4.79
AFFX-GapdhMur/M32599_M_at	GAPDHM_Mm_AFFX	Glyceraldehyde-3-phospate dehydrogenase middle control sequence (M. musculus) [AFFX]	Uterus	4.79
AFFX-GapdhMur/M32599_M_at	GAPDHM_Mm_AFFX	Glyceraldehyde-3-phospate dehydrogenase middle control sequence (M. musculus) [AFFX]	Uterus	4.79
94246_at	ETS2	E26 avian leukemia oncogene 2, 3' domain	Uterus	4.78
98930_at	COPE	coatomer protein complex, subunit epsilon	Uterus	4.76
98928_at	CORO1B	coronin, actin binding protein 1B	Uterus	4.76
94821_at	XBP1	X-box binding protein 1	Uterus	4.69
95708_at	D3UCLA1	DNA segment, Chr 3, University of California at Los Angeles 1	Uterus	4.66
96284_at	UNK_AW121446	ESTs, Moderately similar to CASEIN KINASE I, GAMMA ISOFORM [Bos taurus]	Uterus	4.64
104279_at	UNK_AW125116	ESTs, Highly similar to DNA-DIRECTED RNA POLYMERASE II 14.4 KD POLYPEPTIDE [Homo sapiens; Cricetulus griseus]	Uterus	4.62
93541_at	TAGLN	transgelin	Uterus	4.62
93798_at	LDH1	lactate dehydrogenase 1, A chain	Uterus	4.61
99926_at	PIGR	polymeric immunoglobulin receptor	Uterus	4.61
99338_at	UNK_AA674798	ESTs, Highly similar to TIP120 [R.norvegicus]	Uterus	4.6

TABLE III - Genes Regulated By Estrogen in the Uterus

Mousedata. Qualifier	Pub_Name	Gene Name	Tissue	Mean WT E2 Fold Change
93066_at	GRN	granulin	Uterus	4.6
99366_at	UNK_AI553536	Cluster Incl AI553536:vw39e06.x1 Mus musculus cDNA, 3' end /clone=IMAGE-1246210 /clone_end=3' /gb=AI553536 /gi=4485899 /ug=Mm.5675 /len=408 /STRA=rev	Uterus	4.58
103556_at	UNK_AI840158	Cluster Incl AI840158:UI-M-AO0-acc-d-08-0-UI.s1 Mus musculus cDNA, 3' end /clone=UI-M-AO0-acc-d-08-0-UI /clone_end=3' /gb=AI840158 /gi=5474371 /ug=Mm.19081 /len=406 /STRA=for	Uterus	4.55
101095_at	MFAP2	microfibrillar-associated protein 2	Uterus	4.53
100323_at	AMD2	S-adenosylmethionine decarboxylase 2	Uterus	4.5
102161_f_at	H2-Q2	histocompatibility 2, Q region locus 2	Uterus	4.5
101955_at	HSPA5	heat shock 70kD protein 5 (glucose-regulated protein, 78kD)	Uterus	4.49
95654_at	UNK_AF109905	Cluster Incl AF109905:Mus musculus major histocompatibility locus class III regions Hsc70t gene, partial cds; smRNP, G7A, NG23, MutS homolog, CLCP, NG24, NG25, and NG26 genes, complete cds; and unknown genes /cds=(0,725) /gb=AF109905 /gi=3986751 /ug=Mm.29	Uterus	4.49
98107_at	UNK_AW123801	Cluster Incl AW123801:UI-M-BH2.1-apm-e-08-0-UI.s1 Mus musculus cDNA, 3' end /clone=UI-M-BH2.1-apm-e-08-0-UI /clone_end=3' /gb=AW123801 /gi=6099331 /ug=Mm.34796 /len=367 /STRA=for	Uterus	4.48
92925_at	CEBPB	CCAAT/enhancer binding protein (C/EBP), beta	Uterus	4.47
92625_at	NME2	expressed in non-metastatic cells 2, protein (NM23B) (nucleoside diphosphate kinase)	Uterus	4.46
96283_at	ITM3-PENDING	integral membrane protein 3	Uterus	4.43
97809_at	UNK_AF109906	Cluster Incl AF109906:Mus musculus MHC class III region RD gene, partial cds; Bf, C2, G9A, NG22, G9, HSP70, HSP70, HSC70t, and smRNP genes, complete cds; G7A gene, partial cds; and unknown genes /cds=(0,3002) /gb=AF109906 /gi=3986763 /ug=Mm.28155 /len=300	Uterus	4.4

TABLE III - Genes Regulated By Estrogen in the Uterus

Mousedata. Qualifier	Pub_Name	Gene Name	Tissue	Mean WT E2 Fold Change
103709_at	UNK_AA763466	Cluster Incl AA763466:vw54f05.r1 Mus musculus cDNA, 5' end /clone=IMAGE-1247649 /clone_end=5' /gb=AA763466 /gi=2813213 /ug=Mm.24093 /len=379 /STRA=for	Uterus	4.37
98562_at	C1QA	complement component 1, q subcomponent, alpha polypeptide	Uterus	4.37
92644_s_at	MYB	myeloblastosis oncogene	Uterus	4.37
99624_at	RPL5	ribosomal protein L5	Uterus	4.33
104093_at	LSP1	lymphocyte specific 1	Uterus	4.31
99942_s_at	CNN1	calponin 1	Uterus	4.31
101055_at	PPGB	protective protein for beta-galactosidase	Uterus	4.3
100059_at	CYBA	cytochrome b-245, alpha polypeptide	Uterus	4.29
94868_at	UNK_AW049812	ESTs, Highly similar to GLUTAMINYL-TRNA SYNTHETASE [Homo sapiens]	Uterus	4.28
93751_at	UNK_AW048157	ESTs, Highly similar to PROBABLE UBIQUITIN CARBOXYL-TERMINAL HYDROLASE [Mus musculus]	Uterus	4.27
101061_at	UNK_AI845293	ESTs, Highly similar to TRANSLOCON-ASSOCIATED PROTEIN, BETA SUBUNIT PRECURSOR [Homo sapiens]	Uterus	4.26
101916_at	DHCR7	7-dehydrocholesterol reductase	Uterus	4.25
93327_at	UNK_AI842665	ESTs, Highly similar to HYPOTHETICAL 13.5 KD PROTEIN C45G9.7 IN CHROMOSOME III [Caenorhabditis elegans]	Uterus	4.25
102968_at	GGTLA1	gamma-glutamyltransferase-like activity 1	Uterus	4.24
99106_at	COPS6	COP9 (constitutive photomorphogenic), subunit 6 (Arabidopsis)	Uterus	4.22
97160_at	SPARC	secreted acidic cysteine rich glycoprotein	Uterus	4.22
96943_at	UNK_AW125234	ESTs, Highly similar to FUSCA PROTEIN FUS6 [Arabidopsis thaliana]	Uterus	4.2
97320_at	UNK_AI842734	ESTs, Weakly similar to KE4_MOUSE HISTIDINE-RICH PROTEIN KE4□ [M.musculus]	Uterus	4.18
96353_at	UNK_AW125346	ESTs, Moderately similar to AF151028_1 HSPC194 [H.sapiens]	Uterus	4.17
94854_g_at	GNB1	guanine nucleotide binding protein, beta 1	Uterus	4.15

TABLE III - Genes Regulated By Estrogen in the Uterus

Mousedata. Qualifier	Pub_Name	Gene Name	Tissue	Mean WT E2 Fold Change
97894_at	UNK_AF109905	Cluster Incl AF109905:Mus musculus major histocompatibility locus class III regions Hsc70t gene, partial cds; smRNP, G7A, NG23, MutS homolog, CLCP, NG24, NG25, and NG26 genes, complete cds; and unknown genes /cds=(0,3791) /gb=AF109905 /gi=3986751 /ug=Mm.2	Uterus	4.14
93390_g_at	PROM	prominin	Uterus	4.13
103429_i_at	UNK_AW125330	ESTs, Moderately similar to unnamed protein product [H.sapiens]	Uterus	4.1
96186_at	UNK_AI839286	ESTs, Moderately similar to Unknown [H.sapiens]	Uterus	4.09
103335_at	LGALS9	lectin, galactose binding, soluble 9	Uterus	4.07
101393_at	ANXA3	annexin A3	Uterus	4.07
93389_at	PROM	prominin	Uterus	4.06
103888_at	RBPMS	RNA-binding protein gene with multiple splicing	Uterus	4.06
96258_at	D13ERTD372E	DNA segment, Chr 13, ERATO Doi 372, expressed	Uterus	4.03
95161_at	D10ERTD73E	DNA segment, Chr 10, ERATO Doi 73, expressed	Uterus	4.03
96869_at	GABARAP	gamma-aminobutyric acid receptor associated protein	Uterus	4.02
101558_s_at	PSMB5	proteasome (prosome, macropain) subunit, beta type 5	Uterus	4
96883_at	EIF3S4	eukaryotic translation initiation factor 3, subunit 4 (delta, 44 kDa)	Uterus	3.98
99549_at	OGN	osteoglycin	Uterus	3.95
101781_f_at	UNK_V00754	HISTONE H3.4	Uterus	3.95
93975_at	33 POLYPEPTIDE□ [R.NORVEGICUS]	ESTs, Highly similar to G33_RAT GENE 33 POLYPEPTIDE□ [R.norvegicus]	Uterus	3.91
92930_at	DLX5	distal-less homeobox 5	Uterus	3.91
95462_at	UNK_AW060951	ESTs, Highly similar to unknown [R.norvegicus]	Uterus	3.9
102791_at	PSMB8	proteosome (prosome, macropain) subunit, beta type 8 (large multifunctional protease 7)	Uterus	3.89
95215_f_at	UBC	ubiquitin C	Uterus	3.89
92850_at	UNK_AI836446	ESTs, Moderately similar to KIAA1398 protein [H.sapiens]	Uterus	3.88
100332_s_at	PRDX5-RS3	peroxiredoxin 5, related sequence 3	Uterus	3.87
100561_at	IQGAP1	IQ motif containing GTPase activating protein 1	Uterus	3.84

TABLE III - Genes Regulated By Estrogen in the Uterus

Mousedata. Qualifier	Pub_Name	Gene Name	Tissue	Mean WT E2 Fold Change
98446_s_at	EPHB4	Eph receptor B4	Uterus	3.82
100771_at	LY57	lymphocyte antigen 57	Uterus	3.81
103547_at	UNK_AI837116	Cluster Incl AI837116:UI-M-AK0-adc-e-09-0-UI.s1 Mus musculus cDNA, 3' end /clone=UI-M-AK0-adc-e-09-0-UI /clone_end=3' /gb=AI837116 /gi=5471329 /ug=Mm.23723 /len=323 /STRA=rev	Uterus	3.81
104365_at	SCAMP2	secretory carrier membrane protein 2	Uterus	3.8
93496_at	UNK_AI852098	ESTs, Weakly similar to AF104033_1 MUEL protein [M.musculus]	Uterus	3.79
100970_at	AKT	thymoma viral proto-oncogene	Uterus	3.79
96318_at	D17WSU104E	DNA segment, Chr 17, Wayne State University 104, expressed	Uterus	3.78
93430_at	CMKOR1	chemokine orphan receptor 1	Uterus	3.75
92882_at	RAB1	RAB1, member RAS oncogene family	Uterus	3.74
97824_at	D11ERTD175E	DNA segment, Chr 11, ERATO Doi 175, expressed	Uterus	3.72
99991_at	IL17R	interleukin 17 receptor	Uterus	3.72
100684_at	PRKCSH	protein kinase C substrate 80K-H	Uterus	3.72
96935_at	UNK_AW011791	ESTs, Moderately similar to epithelial protein up-regulated in carcinoma [H.sapiens]	Uterus	3.71
93500_at	ALAS1	aminolevulinic acid synthase 1	Uterus	3.69
100554_at	PDLIM1	PDZ and LIM domain 1 (elfin)	Uterus	3.67
94105_at	CDC42	cell division cycle 42 homolog (S. cerevisiae)	Uterus	3.66
101486_at	PSMB10	proteasome (prosome, macropain) subunit, beta type 10	Uterus	3.66
96155_at	UNK_AW049359	ESTs, Highly similar to AF177476_1 CDK5 activator-binding protein [R.norvegicus]	Uterus	3.65
99475_at	CISH2	cytokine inducible SH2-containing protein 2	Uterus	3.64
102767_at	AA536815	EST AA536815	Uterus	3.64
104315_at	UNK_AI846773	Cluster Incl AI846773:UI-M-AO1-ael-f-02-0-UI.s1 Mus musculus cDNA, 3' end /clone=UI-M-AO1-ael-f-02-0-UI /clone_end=3' /gb=AI846773 /gi=5490679 /ug=Mm.22413 /len=322 /STRA=for	Uterus	3.64
104389_at	UNK_AW049360	ESTs, Weakly similar to T17295 hypothetical protein DKFZp434H132.1 - human [H.sapiens]	Uterus	3.63

TABLE III - Genes Regulated By Estrogen in the Uterus

Mousedata. Qualifier	Pub_Name	Gene Name	Tissue	Mean WT E2 Fold Change
93833_s_at	UNK_X05862	Cluster Incl X05862:Mouse H2B and H2A histone genes (291A) /cds=(0,380) /gb=X05862 /gi=51302 /ug=Mm.21579 /len=381 /STRA=for	Uterus	3.61
101881_g_at	COL18A1	procollagen, type XVIII, alpha 1	Uterus	3.61
100569_at	ANXA2	annexin A2	Uterus	3.6
94561_at	UNK_AI836140	Mus musculus epithelial protein lost in neoplasm-a (Eplin) mRNA, complete cds	Uterus	3.59
95608_at	CTSB	cathepsin B	Uterus	3.57
96709_at	UNK_AI839839	ESTs, Highly similar to EST00098 protein [H.sapiens]	Uterus	3.57
93102_f_at	ACTG2	actin, gamma 2, smooth muscle, enteric	Uterus	3.55
99477_at	GNG12	guanine nucleotide binding protein (G protein), gamma 12	Uterus	3.55
94237_at	D6WSU137E	DNA segment, Chr 6, Wayne State University 137, expressed	Uterus	3.55
98937_at	TBRG1	transforming growth factor beta regulated gene 1	Uterus	3.53
94503_at	UNK_AI842492	ESTs, Highly similar to RAS-RELATED PROTEIN RAB-8 [Homo sapiens; Canis familiaris]	Uterus	3.5
99019_at	POR	P450 (cytochrome) oxidoreductase	Uterus	3.49
104623_at	TLE3	transducin-like enhancer of split 3, homolog of Drosophila E(spl)	Uterus	3.47
92866_at	H2-AA	histocompatibility 2, class II antigen A, alpha	Uterus	3.46
103200_at	UNK_AA711773	Cluster Incl AA711773:vu58g05.r1 Mus musculus cDNA, 5' end /clone=IMAGE-1195640 /clone_end=5' /gb=AA711773 /gi=2721691 /ug=Mm.1902 /len=473 /STRA=for	Uterus	3.44
104100_at	UNK_AI845915	Cluster Incl AI845915:UI-M-AK1-aex-d-02-0-UI.s1 Mus musculus cDNA, 3' end /clone=UI-M-AK1-aex-d-02-0-UI /clone_end=3' /gb=AI845915 /gi=5489821 /ug=Mm.21864 /len=208 /STRA=for	Uterus	3.42
94063_at	SEMA4A	sema domain, immunoglobulin domain (Ig), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 4A	Uterus	3.42
95752_at	UNK_AI837369	ESTs, Highly similar to unnamed protein product [H.sapiens]	Uterus	3.42
97409_at	IFI1	interferon inducible protein 1	Uterus	3.41

TABLE III - Genes Regulated By Estrogen in the Uterus

Mousedata. Qualifier	Pub_Name	Gene Name	Tissue	Mean WT E2 Fold Change
95749_at	UNK_AW122364	ESTs, Highly similar to ARGR_HUMAN ARGININE-RICH PROTEIN□ [H.sapiens]	Uterus	3.41
104110_at	UNK_AW060515	Cluster Incl AW060515:UI-M-BH1-ann-d-07-0-UI.s1 Mus musculus cDNA, 3' end /clone=UI-M-BH1-ann-d-07-0-UI /clone_end=3' /gb=AW060515 /gi=6008266 /ug=Mm.21919 /len=330 /STRA=for	Uterus	3.39
99101_at	EIF3S7	eIF3 p66	Uterus	3.39
94966_at	G6PDX	glucose-6-phosphate dehydrogenase X-linked	Uterus	3.38
92567_at	COL5A2	procollagen, type V, alpha 2	Uterus	3.37
103016_s_at	CD68	CD68 antigen	Uterus	3.36
AFFX-b-ActinMur/M12481_3_at	BACTIN3_Mm_AFFX	Beta-actin 3' control sequence (M. musculus) [AFFX]	Uterus	3.35
AFFX-b-ActinMur/M12481_3_at	BACTIN3_Mm_AFFX	Beta-actin 3' control sequence (M. musculus) [AFFX]	Uterus	3.35
AFFX-b-ActinMur/M12481_3_at	BACTIN3_Mm_AFFX	Beta-actin 3' control sequence (M. musculus) [AFFX]	Uterus	3.35
96653_at	APP	amyloid beta (A4) precursor protein	Uterus	3.35
99872_s_at	FTL1	ferritin light chain 1	Uterus	3.34
97125_f_at	LOC56628	MHC (A.CA/J(H-2K-f) class I antigen	Uterus	3.33
94288_at	HIS1A	histone H1	Uterus	3.32
93276_at	HN1	hematological and neurological expressed sequence 1	Uterus	3.29
93071_at	TIF1B	transcriptional intermediary factor 1, beta	Uterus	3.28
99032_at	RASD1	RAS, dexamethasone-induced 1	Uterus	3.27
100428_at	LAMC2	laminin, gamma 2	Uterus	3.25
103708_at	UNK_AI132207	Cluster Incl AI132207:ue28g02.x1 Mus musculus cDNA, 3' end /clone=IMAGE-1481714 /clone_end=3' /gb=AI132207 /gi=3602223 /ug=Mm.24090 /len=450 /STRA=for	Uterus	3.23
96693_at	UNK_AI849453	ESTs, Highly similar to ARGINYL-TRNA SYNTHETASE [Cricetulus longicaudatus]	Uterus	3.23
94831_at	CTSB	cathepsin B	Uterus	3.2
95493_at	COL6A1	procollagen, type VI, alpha 1	Uterus	3.2

TABLE III - Genes Regulated By Estrogen in the Uterus

Mousedata. Qualifier	Pub_Name	Gene Name	Tissue	Mean WT E2 Fold Change
99562_at	MAN2B1	mannosidase 2, alpha B1	Uterus	3.2
101487_f_at	LY6E	lymphocyte antigen 6 complex, locus E	Uterus	3.18
100081_at	STIP1	stress-induced phosphoprotein 1	Uterus	3.18
94061_at	CRIP	cysteine rich intestinal protein	Uterus	3.18
101060_at	GRP58	glucose regulated protein, 58 kDa	Uterus	3.16
98522_at	PSMD8	proteasome (prosome, macropain) 26S subunit, non-ATPase, 8	Uterus	3.16
101834_at	MAPK3	mitogen activated protein kinase 3	Uterus	3.14
96657_at	SAT	spermidine/spermine N1-acetyl transferase	Uterus	3.14
92632_at	UNK_AI842328	Mus musculus calmodulin III (Calm3) mRNA, 3' untranslated region	Uterus	3.12
99992_at	UNK_AI286698	ESTs, Highly similar to interleukin 17 receptor□ [M.musculus]	Uterus	3.12
94282_at	ASAH1	N-acylsphingosine amidohydrolase 1	Uterus	3.11
94788_f_at	TUBB5	tubulin, beta 5	Uterus	3.11
103398_at	UNK_AW123232	Cluster Incl AW123232:UI-M-BH2.1-apd-g-08-0-UI.s1 Mus musculus cDNA, 3' end /clone=UI-M-BH2.1-apd-g-08-0-UI /clone_end=3' /gb=AW123232 /gi=6098727 /ug=Mm.18714 /len=469 /STRA=rev	Uterus	3.1
95149_at	COPZ1	coatomer protein complex, subunit zeta 1	Uterus	3.1
103891_i_at	UNK_AI197161	ESTs, Moderately similar to ELL2_HUMAN RNA POLYMERASE II ELONGATION FACTOR ELL2□ [H.sapiens]	Uterus	3.09
98104_at	UNK_AI842889	ESTs, Highly similar to PROTEOLIPID PROTEIN PPA1 [Saccharomyces cerevisiae]	Uterus	3.08
102916_s_at	CREBL1	cAMP responsive element binding protein-like 1	Uterus	3.08
101591_at	UNK_AI852589	ESTs, Highly similar to HYPOTHETICAL PROTEIN C22G7.01C IN CHROMOSOME I [Schizosaccharomyces pombe]	Uterus	3.07
100949_at	UNK_AI461767	ESTs, Weakly similar to hypothetical protein [H.sapiens]	Uterus	3.05
96573_at	ACTG	actin, gamma, cytoplasmic	Uterus	3.04
100948_at	D15ERTD221E	DNA segment, Chr 15, ERATO Doi 221, expressed	Uterus	3.03

TABLE III - Genes Regulated By Estrogen in the Uterus

Mousedata. Qualifier	Pub_Name	Gene Name	Tissue	Mean WT E2 Fold Change
101754_f_at	SPRR2G	small proline-rich protein 2G	Uterus	3.02
97829_at	UNK_AI838053	ESTs, Highly similar to phosphatidylinositol synthase [R.norvegicus]	Uterus	3.02
101886_f_at	H2-L	histocompatibility 2, L region	Uterus	3.01
99067_at	GAS6	growth arrest specific 6	Uterus	3
101571_g_at	IGFBP4	insulin-like growth factor binding protein 4	Uterus	3
100998_at	H2-AB1	histocompatibility 2, class II antigen A, beta 1	Uterus	3
96135_at	UNK_AA833425	ESTs, Highly similar to AF161398_1 HSPC280 [H.sapiens]	Uterus	2.99
101105_at	BCRP1-PENDING	breakpoint cluster region protein 1	Uterus	2.97
96024_at	AHCY	S-adenosylhomocysteine hydrolase	Uterus	2.96
100496_at	PAM	peptidylglycine alpha-amidating monooxygenase	Uterus	2.95
103755_at	SH3D19	SH3 domain protein D19	Uterus	2.95
97817_at	SPEC1-PENDING	small protein effector 1 of Cdc42	Uterus	2.95
98543_at	CTSS	cathepsin S	Uterus	2.95
93548_at	UNK_AW122942	ESTs, Highly similar to PROTEIN TRANSPORT PROTEIN SEC61 BETA SUBUNIT [Homo sapiens; Canis familiaris]	Uterus	2.94
97197_r_at	UNK_C78850	Mouse (AKR/J) endogenous retrovirus, clone A-12, pol-env region	Uterus	2.93
102370_at	UNK_AA822174	Cluster Incl AA822174:vp36a09.r1 Mus musculus cDNA, 5' end /clone=IMAGE-1078744 /clone_end=5' /gb=AA822174 /gi=2892042 /ug=Mm.1187 /len=329 /STRA=for	Uterus	2.91
97559_at	EEF2	eukaryotic translation elongation factor 2	Uterus	2.9
96732_at	UNK_AI851081	ESTs, Highly similar to T17338 hypothetical protein DKFZp434O125.1 - human [H.sapiens]	Uterus	2.89
92450_at	SLC12A4	solute carrier family 12, member 4	Uterus	2.88
93126_at	CKB	creatine kinase, brain	Uterus	2.87
98417_at	MX1	myxovirus (influenza virus) resistance 1	Uterus	2.87
96360_at	UNK_AW125498	ESTs, Weakly similar to GDIS_MOUSE RHO GDP-DISSOCIATION INHIBITOR 2 [M.musculus]	Uterus	2.86
96356_at	AF007010	EST AF007010	Uterus	2.84

TABLE III - Genes Regulated By Estrogen in the Uterus

Mousedata. Qualifier	Pub_Name	Gene Name	Tissue	Mean WT E2 Fold Change
95593_at	UNK_AW125446	Cluster Incl AW125446:UI-M-BH2.3-aqh-h-05-0-UI.s1 Mus musculus cDNA, 3' end /clone=UI-M-BH2.3-aqh-h-05-0-UI /clone_end=3' /gb=AW125446 /gi=6100976 /ug=Mm.27902 /len=540 /STRA=for	Uterus	2.84
98405_at	SPI6	serine protease inhibitor 6	Uterus	2.84
102804_at	CEACAM1	CEA-related cell adhesion molecule 1	Uterus	2.83
92836_at	UNK_AA919594	Cluster Incl AA919594:vz22b07.r1 Mus musculus cDNA, 5' end /clone=IMAGE-1316437 /clone_end=5' /gb=AA919594 /gi=3066373 /ug=Mm.13097 /len=222 /STRA=for	Uterus	2.83
100460_at	TSBP	TPR-containing, SH2-binding phosphoprotein	Uterus	2.83
100094_at	SUPT5H	suppressor of Ty 5 homolog (S. cerevisiae)	Uterus	2.82
100064_f_at	GJA1	gap junction membrane channel protein alpha 1	Uterus	2.81
96632_at	MRGX-PENDING	MORF-related gene X	Uterus	2.81
95721_at	MAPKAPK2	MAP kinase-activated protein kinase 2	Uterus	2.8
101948_at	LAMB1-1	laminin B1 subunit 1	Uterus	2.8
101959_r_at	TFDP1	transcription factor Dp 1	Uterus	2.79
97203_at	MLP	MARCKS-like protein	Uterus	2.77
97496_f_at	UNK_AW048944	ESTs, Weakly similar to polymerase I-transcript release factor [M.musculus]	Uterus	2.76
101877_at	SLC31A1	solute carrier family 31, member 1	Uterus	2.76
104221_at	SLC7A5	solute carrier family 7 (cationic amino acid transporter, y+ system), member 5	Uterus	2.75
98019_at	TGFB1I1	transforming growth factor beta 1 induced transcript 1	Uterus	2.75
101510_at	PSME1	protease (prosome, macropain) 28 subunit, alpha	Uterus	2.74
96340_at	UNK_AW124185	ESTs, Highly similar to C214_HUMAN 17.9 KDA MEMBRANE PROTEIN C21ORF4□ [H.sapiens]	Uterus	2.74
96761_at	UNK_AF109906	Cluster Incl AF109906:Mus musculus MHC class III region RD gene, partial cds; Bf, C2, G9A, NG22, G9, HSP70, HSP70, HSC70t, and smRNP genes, complete cds; G7A gene, partial cds; and unknown genes /cds=(0,2123) /gb=AF109906 /gi=3986763 /ug=Mm.29004 /len=212	Uterus	2.72
102990_at	COL3A1	procollagen, type III, alpha 1	Uterus	2.72
94224_s_at	UNK_M74123	Mus musculus (strain C57Bl/6) mRNA sequence	Uterus	2.71

TABLE III - Genes Regulated By Estrogen in the Uterus

Mousedata. Qualifier	Pub_Name	Gene Name	Tissue	Mean WT E2 Fold Change
100621_at	UNK_AI848825	ESTs, Highly similar to RIBONUCLEASE INHIBITOR [Rattus norvegicus]	Uterus	2.7
102752_at	SHYC	selective hybridizing clone	Uterus	2.7
97013_f_at	CYBA	cytochrome b-245, alpha polypeptide	Uterus	2.68
104248_at	UNK_AW227650	ESTs, Highly similar to TRANSLOCON-ASSOCIATED PROTEIN, GAMMA SUBUNIT [Rattus norvegicus]	Uterus	2.68
104701_at	STRA14	stimulated by retinoic acid 14	Uterus	2.68
103648_at	TACSTD2	tumor-associated calcium signal transducer 2	Uterus	2.67
99514_at	UNK_AI835443	ESTs, Highly similar to B-MYC TRANSFORMING PROTEIN [Rattus norvegicus]	Uterus	2.67
97262_at	UNK_AW050305	ESTs, Highly similar to CASEIN KINASE I, DELTA ISOFORM [Homo sapiens]	Uterus	2.66
95694_at	UNK_X70956	M.musculus TOP gene for topoisomerase I, exons 19-21	Uterus	2.66
101078_at	BSG	basigin	Uterus	2.64
95660_at	UNK_AI851815	Mus musculus HSCO mRNA, complete cds	Uterus	2.63
99993_at	ANPEP	alanyl (membrane) aminopeptidase (aminopeptidase N, aminopeptidase M, microsomal aminopeptidase, CD13, p150)	Uterus	2.63
103494_at	UNK_AI047972	ESTs, Weakly similar to CD63_MOUSE CD63 ANTIGEN [M.musculus]	Uterus	2.63
94929_at	PTPN1	protein tyrosine phosphatase, non-receptor type 1	Uterus	2.6
100610_at	CAPN4	calpain 4	Uterus	2.6
97890_at	SGK	serum/glucocorticoid regulated kinase	Uterus	2.6
100889_at	UNK_AI838576	Cluster Incl AI838576:UI-M-AO0-abz-c-02-0-UI.s1 Mus musculus cDNA, 3' end /clone=UI-M-AO0-abz-c-02-0-UI /clone_end=3' /gb=AI838576 /gi=5472789 /ug=Mm.54120 /len=181 /STRA=rev	Uterus	2.59
100475_at	ZFP147	zinc finger protein 147	Uterus	2.59
98946_at	WSB1	WSB-1	Uterus	2.59
96912_s_at	CTLA2A	cytotoxic T lymphocyte-associated protein 2 alpha	Uterus	2.58
96069_at	UNK_AI840094	ESTs, Highly similar to AFLATOXIN B1 ALDEHYDE REDUCTASE [Rattus norvegicus]	Uterus	2.57
100723_f_at	SPRR2E	small proline-rich protein 2E	Uterus	2.57
93058_at	EIF1A	eukaryotic translation initiation factor 1A	Uterus	2.56

TABLE III - Genes Regulated By Estrogen in the Uterus

Mousedata. Qualifier	Pub_Name	Gene Name	Tissue	Mean WT E2 Fold Change
94301_at	ATP6K	ATPase, H+ transporting lysosomal (vacuolar proton pump), 9.2 kDa	Uterus	2.55
93680_at	STK10	serine/threonine kinase 10	Uterus	2.55
93499_at	CAPPA1	capping protein alpha 1	Uterus	2.55
100422_i_at	UNK_AJ237939	Cluster Incl AJ237939:Mus musculus partial STAT5B gene, exons 6-9 /cds=(0,618) /gb=AJ237939 /gi=5689871 /ug=Mm.4697 /len=619 /STRA=for	Uterus	2.53
96333_g_at	UNK_AW259199	ESTs, Weakly similar to AF154120_1 sorting nexin 1 [M.musculus]	Uterus	2.53
103918_at	SLC15A2	solute carrier family 15 (H+/peptide transporter), member 2	Uterus	2.53
101982_at	VASP	vasodilator-stimulated phosphoprotein	Uterus	2.53
104155_f_at	ATF3	activating transcription factor 3	Uterus	2.53
96633_s_at	MRGX-PENDING	MORF-related gene X	Uterus	2.52
95397_at	UNK_AI852661	Cluster Incl AI852661:UI-M-BH0-aji-a-10-0-UI.s1 Mus musculus cDNA, 3' end /clone=UI-M-BH0-aji-a-10-0-UI /clone_end=3' /gb=AI852661 /gi=5496567 /ug=Mm.2388 /len=297 /STRA=for	Uterus	2.5
92809_r_at	FKBP4	FK506 binding protein 4 (59 kDa)	Uterus	2.5
100136_at	LAMP2	lysosomal membrane glycoprotein 2	Uterus	2.5
93250_r_at	HMGB2	high mobility group box 2	Uterus	2.48
103551_at	AI428202	EST AI4282022	Uterus	2.48
100686_at	LLREP3	repeat family 3 gene	Uterus	2.46
92256_at	CTSB	cathepsin B	Uterus	2.46
92226_at	UNK_AA866971	ESTs, Moderately similar to hypothetical protein [H.sapiens]	Uterus	2.45
96056_at	ARHC	aplysia ras-related homolog 9 (RhoC)	Uterus	2.45
96920_at	PRSS11	insulin-like growth factor binding protein 5 protease	Uterus	2.44
101019_at	CTSC	cathepsin C	Uterus	2.44
100600_at	CD24A	CD24a antigen	Uterus	2.44
94915_at	PPIB	peptidylprolyl isomerase B	Uterus	2.44
93323_at	PLP2	proteolipid protein 2	Uterus	2.43

TABLE III - Genes Regulated By Estrogen in the Uterus

Mousedata. Qualifier	Pub_Name	Gene Name	Tissue	Mean WT E2 Fold Change
97386_at	UNK_AI853294	Cluster Incl AI853294:UI-M-BH0-ajl-f-03-0-UI.s1 Mus musculus cDNA, 3' end /clone=UI-M-BH0-ajl-f-03-0-UI /clone_end=3' /gb=AI853294 /gi=5497200 /ug=Mm.29789 /len=413 /STRA=for	Uterus	2.43
96939_at	TRRP2	transient receptor protein 2	Uterus	2.43
95683_g_at	DDB1	damage specific DNA binding protein 1 (127 kDa)	Uterus	2.42
104292_at	EYA2	eyes absent 2 homolog (Drosophila)	Uterus	2.42
104300_at	IQGAP1	IQ motif containing GTPase activating protein 1	Uterus	2.42
95120_at	UNK_AI837621	ESTs, Highly similar to tetraspan NET-6 [H.sapiens]	Uterus	2.41
98059_s_at	LMNA	lamin A	Uterus	2.4
93320_at	CPT1A	carnitine palmitoyltransferase 1, liver	Uterus	2.39
94260_at	UNK_AI850352	ESTs, Moderately similar to KIAA0731 protein [H.sapiens]	Uterus	2.39
94238_at	UNK_AW228316	ESTs, Highly similar to serine protease [H.sapiens]	Uterus	2.39
94206_at	UNK_AC002397	Cluster Incl AC002397:Mouse chromosome 6 BAC-284H12 (Research Genetics mouse BAC library) complete sequence /cds=(108,488) /gb=AC002397 /gi=3287367 /ug=Mm.22195 /len=568 /STRA=for	Uterus	2.38
93336_at	UNK_AW121539	ESTs, Weakly similar to ENDOSOMAL P24B PROTEIN PRECURSOR [Saccharomyces cerevisiae]	Uterus	2.38
94060_at	UNK_AI852623	ESTs, Weakly similar to Edp1 protein [M.musculus]	Uterus	2.38
94834_at	CTSH	cathepsin H	Uterus	2.37
101029_f_at	ACTC1	actin, alpha, cardiac	Uterus	2.37
100928_at	FBLN2	fibulin 2	Uterus	2.37
92769_at	TSTAP91A	tissue specific transplantation antigen P91A	Uterus	2.36
96829_at	D19WSU162E	DNA segment, Chr 19, Wayne State University 162, expressed	Uterus	2.36
93309_at	FIN14	fibroblast growth factor inducible 14	Uterus	2.36
101054_at	II	Ia-associated invariant chain	Uterus	2.35
94839_at	NUCB	nucleobindin	Uterus	2.35
98437_at	CASP3	caspase 3, apoptosis related cysteine protease	Uterus	2.34
98465_f_at	IFI204	interferon activated gene 204	Uterus	2.33
98463_at	REGULATOR [DROSOPHILA MELANOGASTER]	ESTs, Highly similar to HOMEOTIC GENE REGULATOR [Drosophila melanogaster]	Uterus	2.33

TABLE III - Genes Regulated By Estrogen in the Uterus

Mousedata. Qualifier	Pub_Name	Gene Name	Tissue	Mean WT E2 Fold Change
103399_at	SCML1	sex comb on midleg-like 1 (Drosophila)	Uterus	2.32
94327_at	UNK_AW230209	ESTs, Moderately similar to unnamed protein product [H.sapiens]	Uterus	2.31
96345_at	D2UCLA1	DNA segment, Chr 2, University of California at Los Angeles 1	Uterus	2.31
97751_f_at	UNK_AI835771	ESTs, Moderately similar to G3P_MOUSE GLYCERALDEHYDE 3-PHOSPHATE DEHYDROGENASE [M.musculus]	Uterus	2.3
101047_at	VIM	vimentin	Uterus	2.3
100772_g_at	LY57	lymphocyte antigen 57	Uterus	2.3
95128_at	NCOR2	nuclear receptor co-repressor 2	Uterus	2.29
93101_s_at	NEDD4	neural precursor cell expressed, developmentally down-regulated gene 4	Uterus	2.29
99119_at	CFL1	cofilin 1, non-muscle	Uterus	2.29
103341_at	CTPS	cytidine 5'-triphosphate synthase	Uterus	2.28
92603_at	ATP6D	ATPase, H+ transporting, lysosomal (vacuolar proton pump), 42 kDa	Uterus	2.28
96708_at	UNK_AW120643	ESTs, Highly similar to COP-COATED VESICLE MEMBRANE PROTEIN P24 PRECURSOR [Cricetulus griseus]	Uterus	2.28
99100_at	STAT3	signal transducer and activator of transcription 3	Uterus	2.28
95105_at	UNK_AI847697	ESTs, Weakly similar to AF077034_1 HSPC010 [H.sapiens]	Uterus	2.27
95142_s_at	CAPPB1	capping protein beta 1	Uterus	2.27
94522_at	DCTN3	dynactin 3	Uterus	2.26
98472_at	H2-T23	histocompatibility 2, T region locus 23	Uterus	2.24
96025_g_at	AHCY	S-adenosylhomocysteine hydrolase	Uterus	2.24
97689_at	F3	coagulation factor III	Uterus	2.23
104533_at	UNK_AA764261	ESTs, Weakly similar to myelin transcription factor 1-like [M.musculus]	Uterus	2.23
104669_at	IRF7	interferon regulatory factor 7	Uterus	2.21
97885_at	1810009M01RIK	RIKEN cDNA 1810009M01 gene	Uterus	2.21
92616_at	UBE1X	ubiquitin-activating enzyme E1, Chr X	Uterus	2.21
93046_at	NUP50	nucleoprotein 50	Uterus	2.2

TABLE III - Genes Regulated By Estrogen in the Uterus

Mousedata. Qualifier	Pub_Name	Gene Name	Tissue	Mean WT E2 Fold Change
98608_at	D6ERTD109E	DNA segment, Chr 6, ERATO Doi 109, expressed	Uterus	2.19
92909_at	PGF	placental growth factor	Uterus	2.19
101009_at	KRT2-8	keratin complex 2, basic, gene 8	Uterus	2.19
100154_at	D17WSU91E	DNA segment, Chr 17, Wayne State University 91, expressed	Uterus	2.19
96658_at	UNK_AI841906	Cluster Incl AI841906:UI-M-AO0-acd-e-10-0-UI.s1 Mus musculus cDNA, 3' end /clone=UI-M-AO0-acd-e-10-0-UI /clone_end=3' /gb=AI841906 /gi=5476119 /ug=Mm.27344 /len=417 /STRA=for	Uterus	2.18
94018_at	UBL3	ubiquitin-like 3	Uterus	2.18
98129_at	ESET	ERG-associated protein	Uterus	2.17
98498_at	CASP7	caspase 7	Uterus	2.17
94247_at	ETS2	E26 avian leukemia oncogene 2, 3' domain	Uterus	2.16
100084_at	VIL2	villin 2	Uterus	2.15
93093_at	MCL1	myeloid cell leukemia sequence 1	Uterus	2.15
95109_at	UNK_AW121447	ESTs, Weakly similar to SIK similar protein [M.musculus]	Uterus	2.15
101963_at	CTSL	cathepsin L	Uterus	2.14
102821_s_at	RASL2-9	RAS-like, family 2, locus 9	Uterus	2.13
97240_g_at	D19ERTD721E	DNA segment, Chr 19, ERATO Doi 721, expressed	Uterus	2.13
94257_at	ARHGDIB	rho, GDP dissociation inhibitor (GDI) beta	Uterus	2.12
101543_f_at	TUBA6	tubulin alpha 6	Uterus	2.11
100720_at	PABPC1	poly A binding protein, cytoplasmic 1	Uterus	2.11
100566_at	IGFBP5	insulin-like growth factor binding protein 5	Uterus	2.1
95647_f_at	UNK_AI465845	ESTs, Moderately similar to unnamed protein product [H.sapiens]	Uterus	2.1
94899_at	RHOIP3-PENDING	Rho interacting protein 3	Uterus	2.09
104716_at	RBP1	retinol binding protein 1, cellular	Uterus	2.08
96338_at	UNK_AW125059	ESTs, Weakly similar to A53770 growth factor-responsive protein, vascular smooth muscle - rat□ [R.norvegicus]	Uterus	2.08
103350_at	PSMD7	proteasome (prosome, macropain) 26S subunit, non-ATPase, 7	Uterus	2.08
94040_at	ERH	enhancer of rudimentary homolog (Drosophila)	Uterus	2.08

TABLE III - Genes Regulated By Estrogen in the Uterus

Mousedata. Qualifier	Pub_Name	Gene Name	Tissue	Mean WT E2 Fold Change
104041_at	UNK_AW122255	ESTs, Moderately similar to T00076 hypothetical protein KIAA0462 - human [H.sapiens]	Uterus	2.07
96834_at	UNK_AI843586	ESTs, Highly similar to PRE-MRNA SPLICING FACTOR SF2, P33 SUBUNIT [Homo sapiens]	Uterus	2.07
103955_at	UNK_AW050325	ESTs, Highly similar to LAMBDA-CRYSTALLIN [Oryctolagus cuniculus]	Uterus	2.07
93997_at	IFRG15	interferon alpha responsive protein (15 kDa)	Uterus	2.06
99985_at	TXNRD1	thioredoxin reductase 1	Uterus	2.06
104125_at	HA1R-PENDING	Hoxa1 regulated gene	Uterus	2.05
92816_r_at	EIF4A1	eukaryotic translation initiation factor 4A1	Uterus	2.05
98993_at	PPP2R5C	protein phosphatase 2, regulatory subunit B (B56), gamma isoform	Uterus	2.04
98113_at	PSMB1	proteasome (prosome, macropain) subunit, beta type 1	Uterus	2.04
99566_at	TPI	triosephosphate isomerase	Uterus	2.04
101107_at	CALU	calumenin	Uterus	2.04
99599_s_at	UNK_AW210320	ESTs, Weakly similar to AF121217_1 pro-alpha-2(I) collagen [R.norvegicus]	Uterus	2.03
96724_r_at	D17H6S56E-5	DNA segment, Chr 17, human D6S56E 5	Uterus	2.03
97994_at	TCF7	transcription factor 7, T-cell specific	Uterus	2.03
95102_at	UNK_AW123754	ESTs, Moderately similar to APB3_RAT AMYLOID BETA A4 PRECURSOR PROTEIN-BINDING FAMILY A MEMBER 3 [R.norvegicus]	Uterus	2.02
94454_at	PRTB	proline rich protein expressed in brain	Uterus	2.02
103059_at	FXYD3	FXYD domain-containing ion transport regulator 3	Uterus	2.02
93037_i_at	ANXA1	annexin A1	Uterus	2.01
104385_i_at	UNK_AI843901	Cluster Incl AI843901:UI-M-AK1-aeu-g-04-0-UI.s1 Mus musculus cDNA, 3' end /clone=UI-M-AK1-aeu-g-04-0-UI /clone_end=3' /gb=AI843901 /gi=5478114 /ug=Mm.227 /len=300 /STRA=for	Uterus	2.01
93490_at	UNK_AI841771	ESTs, Weakly similar to contains similarity to Saccharomyces cerevisiae MAF1 protein [C.elegans]	Uterus	2
95406_at	UNK_AW125347	Cluster Incl AW125347:UI-M-BH2.1-apy-h-03-0-UI.s1 Mus musculus cDNA, 3' end /clone=UI-M-BH2.1-apy-h-03-0-UI /clone_end=3' /gb=AW125347 /gi=6100877 /ug=Mm.24219 /len=331 /STRA=for	Uterus	1.99

TABLE III - Genes Regulated By Estrogen in the Uterus

Mousedata. Qualifier	Pub_Name	Gene Name	Tissue	Mean WT E2 Fold Change
REPRESSIONS				
93594_r_at	EMP3	epithelial membrane protein 3	Uterus	0.55
104235_at	VAMP2	vesicle-associated membrane protein 2	Uterus	0.54
97317_at	ENPP2	ectonucleotide pyrophosphatase/phosphodiesterase 2	Uterus	0.52
94813_at	GAS1	growth arrest specific 1	Uterus	0.51
95133_at	ASNS	asparagine synthetase	Uterus	0.48
103353_f_at	CYP4B1	cytochrome P450, subfamily IV B, polypeptide 1	Uterus	0.46
99577_at	KITL	kit ligand	Uterus	0.45
102395_at	PMP22	peripheral myelin protein, 22 kDa	Uterus	0.44
93013_at	IDB2	inhibitor of DNA binding 2	Uterus	0.44
101152_at	HTR5A	5-hydroxytryptamine (serotonin) receptor 5A	Uterus	0.41
92589_at	UNK_AI846545	ESTs, Highly similar to SERB_HUMAN L-3-PHOSPHOSERINE PHOSPHATASE [H.sapiens]	Uterus	0.4
104217_at	UNK_AW045753	Cluster Incl AW045753:UI-M-BH1-akt-a-10-0-UI.s1 Mus musculus cDNA, 3' end /clone=UI-M-BH1-akt-a-10-0-UI /clone_end=3' /gb=AW045753 /gi=5906282 /ug=Mm.27893 /len=407 /STRA=rev	Uterus	0.39
93503_at	SDF5	stromal cell derived factor 5	Uterus	0.38
96672_at	UNK_AW123564	ESTs, Weakly similar to S36166 paired box transcription factor Pax-6 - rat [R.norvegicus]	Uterus	0.38
93543_f_at	GSTM1	glutathione S-transferase, mu 1	Uterus	0.36
93836_at	BNIP3	BCL2/adenovirus E1B 19 kDa-interacting protein 1, NIP3	Uterus	0.35
98575_at	FASN	fatty acid synthase	Uterus	0.33
99671_at	ADN	adipsin	Uterus	0.33
101990_at	LDH2	lactate dehydrogenase 2, B chain	Uterus	0.3
98588_at	FAH	fumarylacetoacetate hydrolase	Uterus	0.3
92592_at	GDC1	glycerol phosphate dehydrogenase 1, cytoplasmic adult	Uterus	0.3
104313_at	UNK_AI842432	ESTs, Moderately similar to PHOSPHOGLUCOMUTASE [Rattus norvegicus]	Uterus	0.3
102094_f_at	GSTM1	glutathione S-transferase, mu 1	Uterus	0.3

TABLE III - Genes Regulated By Estrogen In the Uterus

Mousedata. Qualifier	Pub_Name	Gene Name	Tissue	Mean WT E2 Fold Change
92202_g_at	UNK_AI553024	ESTs, Highly similar to 2118318A promyelocyte leukemia Zn finger protein [M.musculus]	Uterus	0.29
94056_at	SCD1	stearoyl-Coenzyme A desaturase 1	Uterus	0.27
95731_at	UNK_AI843106	ESTs, Highly similar to p53 regulated PA26-T2 nuclear protein [H.sapiens]	Uterus	0.27
97844_at	RGS2	regulator of G-protein signaling 2	Uterus	0.26
94516_f_at	PENK2	preproenkephalin 2	Uterus	0.19
95082_at	IGFBP3	insulin-like growth factor binding protein 3	Uterus	0.19
94057_g_at	SCD1	stearoyl-Coenzyme A desaturase 1	Uterus	0.19
101560_at	EMB	embigin	Uterus	0.18
93996_at	CYP2E1	cytochrome P450, 2e1, ethanol inducible	Uterus	0.18
101991_at	FMO1	flavin containing monooxygenase 1	Uterus	0.17
92877_at	TGFBI	transforming growth factor, beta induced, 68 kDa	Uterus	0.16
97402_at	TEMT	thioether S-methyltransferase	Uterus	0.15
100567_at	FABP4	fatty acid binding protein 4, adipocyte	Uterus	0.14
99104_at	ACRP30	adipocyte complement related protein of 30 kDa	Uterus	0.13

TABLE IV - Genes Regulated By Estrogen in the Kidney

							Study 1		Study 1		Study 2				Study 2			
						Approx	WT		ERbKO		WT				ERbKO			
Potential	Potential					Ave	Veh	E2	Veh	E2	Veh	Veh	E2	E2	Veh	Veh	E2	E2
ERa regs	ERb regs	Fragment Name	Exemplar Seq: A	Unigene	Known Gene.Name	Fold	Expr (/evansm/ Kidney, mouse/Study 1, U74v2/WT Vehicle kidney (81010))	Expr (/evansm/Kidney, mouse/Study 1, U74v2/WT E2 kidney (81036))	Expr (/evansm/ Kidney, mouse/Study 1, U74v2/KO Vehicle kidney (81025))	Expr (/evansm/ Kidney, mouse/Study 1, U74v2/KO E2 kidney (81038))	Expr (/evansm/Kidney, mouse/Study 2, U74v2/WT kidney vehicle (82406))	Expr (/evansm/ Kidney, mouse/Study 2, U74v2/WT kidney vehicle (82407))	Expr (/evansm/ Kidney, mouse/Study 2, U74v2/WT kidney E2 (82408))	Expr (/evansm/Kidney, mouse/Study 2, U74v2/WT kidney E2 (82409))	Expr (/evansm/ Kidney, mouse/Study 2, U74v2/ERbKO kidney vehicle (82410))	Expr (/evansm/ Kidney, mouse/Study 2, U74v2/ERbKO kidney vehicle (82411))	Expr (/evansm/ Kidney, mouse/Study 2, U74v2/ERbKO kidney E2 (82412))	
INDUCTIONS																		
x		100060_i_at	M13500		kallikrein 6	15.78	5	39	2	37	1	2	40	31	2	3	44	37
x		100061_f_at	M13500		kallikrein 6	83.25	6	341	3	370	5	4	338	201	3	4	359	297
x		95775_f_at	V00829		kallikrein 1	61.00	7	370	8	333	9	6	345	234	2	4	361	291
x		104495_f_at	Y00500	Mm.30375	kallikrein 5	52.59	5	115	2	125	1	2	145	69	2	3	183	130
x		94716_f_at	M17962	Mm.200410	kallikrein 9	44.67	16	380	8	372	10	10	326	230	3	5	368	302
x		100422_i_at	AJ237939	Mm.4697	signal transducer and activator of transcription 5A	39.21	5	151	2	70	1	2	104	73	2	3	104	92
x		100423_f_at	AJ237939	Mm.4697	signal transducer and activator of transcription 5A	3.80	41	96	13	61	20	14	80	44	15	14	58	72
x		101269_f_at	M17979			28.73	18	305	11	282	15	13	264	178	4	5	295	236
x		100681_f_at	V00829			25.24	8	123	6	122	5	6	142	89	3	3	154	117
x		94773_at	X01801		nerve growth factor, alpha	17.80	5	53	2	59	1	2	30	24	2	3	33	42
x		104497_f_at	X03994	Mm.5193	kallikrein 8	17.44	6	53	3	67	2	2	45	26	2	3	56	49
x		168876_f_at	AV044014	Mm.143833	kallikrein 21	17.40	6	75	4	96	11	7	76	68	4	2	83	70
x		102693_f_at	J00389	Mm.143842	kallikrein 13,kallikrein 26	17.24	5	86	6	79	5	4	70	38	2	3	86	66
x		100719_f_at	J03877	Mm.19214	kallikrein 16	14.45	13	119	7	103	6	7	87	50	3	5	95	82
x		114903_at	AI447633	Mm.26357		13.58	10	96	7	94	11	7	92	97	6	4	126	84
x		139531_at	AW120795	Mm.45188		12.37	2	40	2	24	2	2	32	17	4	2	40	22
x		101870_at	V00793			7.39	5	21	2	14	1	2	18	13	2	3	24	22
x		104221_at	AB017189	Mm.27943	solute carrier family 7 (cationic amino acid transporter, y+ system), member 5	6.99	8	61	9	47	14	8	64	57	4	9	61	61
x		95905_at	AI118078	Mm.24361		6.67	16	83	7	38	6	4	35	30	4	5	46	33
x		111046_r_at	AI957367	Mm.26838	17b dehydrogenase A homolog	6.34	2	17	2	13	3	3	11	9	3	2	12	11
x		133672_at	AI451032	Mm.32389		5.91	2	11	2	16	2	2	15	8	4	2	23	14
x		117208_at	AI838208	Mm.41330	RIKEN cDNA 1110003O08 gene	5.86	3	37	3	18	9	4	16	15	9	4	15	26
x		98591_at	U24703	Mm.3057	reelin	5.28	5	43	3	10	3	3	14	7	2	3	14	12
x		103048_at	M12731	Mm.16469	neuroblastoma myc-related oncogene 1	4.76	6	39	2	9	2	2	9	5	2	3	16	10
x		165248_f_at	AV035328	Mm.37203	placental lactogen 2	4.50	6	38	6	29	4	7	14	32	6	7	27	17
x		92232_at	U88328	Mm.3468	cytokine inducible SH2-containing protein 3	4.37	5	45	6	11	6	5	12	13	7	5	21	15
x		164520_f_at	AV302474	Mm.25743	Tmprss2 Transmembrane protease, serine 2	4.26	1	6	1	8	3	2	7	6	3	3	10	8
x		99632_at	U83902	Mm.43444	MAD2 (mitotic arrest deficient, homolog)-like 1 (yeast)	3.99	9	27	4	16	5	5	19	14	3	5	23	19
x		116273_at	AW123862	Mm.31953		3.91	5	7	5	30	5	4	22	18	5	2	16	13
x		131226_at	AI842542	Mm.23157		3.60	2	8	2	11	2	3	11	8	4	2	8	6
x		104550_at	AW123273	Mm.23710	?CYP2S1 Cytochrome P450	3.54	7	22	5	11	2	3	14	7	3	4	18	10
x		105737_at	AI851277	Mm.39735		3.38	2	8	1	4	2	3	6	3	2	1	8	6
x		100407_at	L38580	Mm.4655	galanin	3.38	24	98	13	42	14	13	41	25	10	11	44	37
x		97353_at	AI837497	Mm.29629	AF8Q34 NGAP-like protein	3.35	13	29	4	14	5	6	16	12	4	5	27	16
x		163941_at	AI846761	Mm.202077	RIKEN cDNA 1110018J23 gene	3.32	31	71	15	60	18	17	55	41	10	12	55	43
x		162976_at	AI838662	Mm.198767	RIKEN cDNA 2700007F12 gene	3.30	3	10	2	8	3	4	9	10	3	2	9	11
x		101979_at	AF055638	Mm.9853	growth arrest and DNA-damage-inducible 45 gamma	3.26	8	42	8	10	6	4	17	10	4	5	19	18
x		93374_at	AI836349	Mm.143762	junctophilin 3	3.23	25	65	6	30	7	10	24	19	8	8	26	19
x		107629_at	AW048768	Mm.27667		3.17	14	34	13	38	8	13	31	36	6	9	29	36
x		112320_at	AI852394	Mm.37753		3.15	14	30	8	30	7	7	16	13	4	6	26	22
x		133278_at	AI452199	Mm.31771		3.11	3	8	2	10	3	3	9	11	6	3	4	10
x		95109_at	AW121447	Mm.29383	NOL5A Nucleolar protein 5A	3.07	33	91	21	69	28	21	74	42	15	20	76	57
x		133815_at	AU042854	Mm.26783		3.01	3	9	2	11	2	2	5	4	5	3	7	8
x		114394_at	AW121080	Mm.32795		2.96	4	17	7	13	5	5	13	9	3	3	11	12
x		167989_at	AA982630	Mm.87051	Weakly similar to high mobility group 1 protein	2.92	5	16	2	7	3	4	8	8	6	4	18	17
x		100938_at	M31658	Mm.144157	growth hormone releasing hormone	2.88	9	41	7	19	8	8	14	13	4	6	11	15
x		97825_at	AI854029	Mm.28209	p53 apoptosis effector related to Pmp22	2.85	10	33	3	12	6	4	9	4	3	5	11	10

TABLE IV - Genes Regulated By Estrogen in the Kidney

							Study 1		Study 1		Study 2				Study 2			
						Approx	WT		ERbKO		WT				ERbKO			
Potential	Potential					Ave	Veh	E2	Veh	E2	Veh	Veh	E2	E2	Veh	Veh	E2	E2
ERa regs	ERb regs	Fragment Name	Exemplar Seq: A	Unigene	Known Gene.Name	Fold	Expr (/evansm/ Kidney, mouse/Study 1, U74v2/WT Vehicle kidney (81010))	Expr (/evansm/Kidney, mouse/Study 1, U74v2/WT E2 kidney (81036))	Expr (/evansm/ Kidney, mouse/Study 1, U74v2/KO Vehicle kidney (81025))	Expr (/evansm/ Kidney, mouse/Study 1, U74v2/KO E2 kidney (81038))	Expr (/evansm/Kidney, mouse/Study 2, U74v2/WT kidney vehicle (82406))	Expr (/evansm/ Kidney, mouse/Study 2, U74v2/WT kidney vehicle (82407))	Expr (/evansm/ Kidney, mouse/Study 2, U74v2/WT kidney E2 (82408))	Expr (/evansm/Kidney, mouse/Study 2, U74v2/WT kidney E2 (82409))	Expr (/evansm/ Kidney, mouse/Study 2, U74v2/ERbKO kidney vehicle (82410))	Expr (/evansm/ Kidney, mouse/Study 2, U74v2/ERbKO kidney vehicle (82411))	Expr (/evansm/ Kidney, mouse/Study 2, U74v2/ERbKO kidney E2 (82412))	
x		136270_at	AI854101	Mm.40241	Highly similar to CRFB MOUSE CORTICOTROPIN-RELEASING FACTOR BINDING PROTEIN PRECURSOR	2.84	3	12	2	7	2	2	7	1	4	2	7	4
x		134303_at	AI553493	Mm.35319		2.81	3	11	3	14	5	6	9	10	5	7	10	9
x		107993_at	AI847249	Mm.27680		2.81	2	9	2	7	3	3	4	4	3	2	5	2
x		104776_at	AA600617	Mm.33238		2.70	1	4	1	4	2	2	6	2	3	1	4	5
x		171390_i_at	AV299689	Mm.21070		2.66	2	3	2	11	2	2	9	6	4	2	4	3
x		103556_at	AI840158	Mm.19081	angiopoietin-like 2	2.60	8	24	7	14	3	2	7	5	3	4	10	11
x		109176_at	AI846059	Mm.29219	RAI17 Retinoic acid induced 17	2.58	47	79	33	104	33	29	75	52	15	22	70	61
x		163186_at	AI852882	Mm.23230	RIKEN cDNA 2610510B01 gene	2.57	20	61	25	50	23	18	42	30	13	16	50	53
x		170686_f_at	AV362687	Mm.89845		2.52	22	58	27	66	11	15	34	23	10	14	38	32
x		164051_at	AV359458	Mm.17850		2.46	87	185	90	201	61	62	132	117	36	52	152	140
x		108749_at	AA611885	Mm.23047		2.41	16	34	12	25	7	6	16	13	5	7	23	21
x		96629_at	X04097	Mm.27194	similar to TESTOSTERONE-REGULATED RP2 PROTEIN	2.32	22	55	11	24	20	19	31	23	6	12	33	26
x		110767_r_at	AA959550	Mm.31540	vascular endothelial growth factor	2.31	13	37	8	17	4	4	11	4	4	4	11	9
x		165772_at	AI851899	Mm.41409	RIKEN cDNA 0610039J01 gene	2.24	17	29	13	26	11	11	21	12	6	6	25	18
x		99552_at	U79550	Mm.4272	slug, chicken homolog	2.23	7	19	6	15	8	7	11	6	4	5	13	12
x		98437_at	U53720	Mm.34405	caspase 3, apoptosis related cysteine protease	2.22	21	39	11	22	10	9	22	18	6	11	27	23
x		165710_at	AA815844		sodium channel, nonvoltage-gated 1 gamma	2.22	3	9	2	6	3	3	5	3	4	3	6	5
x		110591_at	AA270831	Mm.2454	SH3 domain protein D19	2.21	11	23	10	24	8	7	14	11	7	7	22	16
x		113125_at	AI851671	Mm.34064	signal transducer and activator of transcription 5B	2.20	11	31	11	13	10	8	11	9	6	3	13	19
x		110850_at	AA959574	Mm.74711	NRIP1 Nuclear receptor interacting protein 1, RIP140	2.18	28	76	25	47	22	19	32	23	13	18	47	43
x		103739_at	AW230977	Mm.24411	RIKEN cDNA 1110017N23 gene	2.16	10	33	10	14	7	6	11	8	3	7	16	13
x		165032_i_at	AV365668	Mm.200980	RIKEN cDNA 4933429H19 gene	2.15	20	42	12	30	12	5	17	7	9	6	24	15
x		166843_at	AI851523	Mm.200318		2.12	149	174	105	265	83	80	175	174	85	49	181	174
x		166021_at	AI481830	Mm.49448		2.09	7	12	5	14	7	4	11	7	5	4	11	10
x		100440_f_at	U76758	Mm.4789	ankyrin 1, erythroid	2.08	60	119	19	53	20	25	41	39	30	23	47	47
x		163370_at	AI591488	Mm.31024	Osbpl3 Oxysterol binding protein-like 3	2.08	6	10	7	13	5	4	10	8	4	4	14	9
x		96215_f_at	AI153421	Mm.218360		2.08	92	206	32	101	89	80	117	87	46	50	101	66
x		165868_f_at	AV291803	Mm.70127	ribosomal protein L12	2.08	22	40	17	35	20	17	33	25	7	12	31	24
x		98628_f_at	AF003695	Mm.3879	hypoxia inducible factor 1, alpha subunit	1.97	129	288	68	136	88	76	143	100	56	68	153	114
REPRESSIONS																		
x		104880_at	AI843154	Mm.33730		0.50	10	2	11	9	26	13	9	6	18	5	9	5
x		129147_r_at	AI931798	Mm.214530	similar to TYROSINE-PROTEIN KINASE RECEPTOR TIE-1 PRECURSOR	0.50	20	6	15	7	12	7	6	4	6	6	5	4
x		102788_s_at	U70132	Mm.1385	paired-like homeodomain transcription factor 2	0.49	83	23	35	28	41	28	15	7	18	27	15	10
x		166849_at	AI853080	Mm.40716		0.49	40	16	29	17	22	15	8	4	11	13	9	7
x		165678_i_at	AI482191	Mm.33178		0.49	23	11	15	8	15	14	6	4	10	12	7	6
x		108195_at	AI851948	Mm.22806		0.48	17	8	15	7	12	9	5	4	7	8	5	4
x		131149_at	AW214502	Mm.27650	RIKEN cDNA 5033417D07 gene	0.48	17	7	16	7	14	13	6	6	10	11	6	7
x		162645_at	AI851427	Mm.25594	protein kinase, cAMP dependent regulatory, type II beta	0.48	20	11	15	8	14	12	4	2	10	11	6	5
x		100539_at	AI841279	Mm.157073	?HBACH Cytosolic acyl coenzyme A thioester hydrolase	0.47	38	16	22	13	22	19	6	3	7	19	8	10
x		169068_i_at	AV206068	Mm.59239	RIKEN cDNA 4930434J08 gene	0.46	6	2	8	4	3	3	2	1	4	5	2	2
x		99127_at	X61506	Mm.4098	spinocerebellar ataxia 10 homolog (human)	0.45	136	66	62	28	53	43	16	7	29	50	21	16
x		108265_at	AW120464	Mm.54156		0.45	32	12	16	8	24	27	11	7	17	20	12	7

TABLE IV - Genes Regulated By Estrogen in the Kidney

							Study 1		Study 1		Study 2				Study 2			
						Approx	WT		ERbKO		WT				ERbKO			
Potential	Potential					Ave	Veh	E2	Veh	E2	Veh	Veh	E2	E2	Veh	Veh	E2	E2
ERa regs	ERb regs	Fragment Name	Exemplar Seq: A	Unigene	Known Gene.Name	Fold	Expr (/evansm/ Kidney, mouse/Study 1, U74v2/WT Vehicle kidney (81010))	Expr (/evansm/Kidney, mouse/Study 1, U74v2/WT E2 kidney (81036))	Expr (/evansm/ Kidney, mouse/Study 1, U74v2/KO Vehicle kidney (81025))	Expr (/evansm/ Kidney, mouse/Study 1, U74v2/KO E2 kidney (81038))	Expr (/evansm/Kidney, mouse/Study 2, U74v2/WT kidney vehicle (82406))	Expr (/evansm/ Kidney, mouse/Study 2, U74v2/WT kidney vehicle (82407))	Expr (/evansm/ Kidney, mouse/Study 2, U74v2/WT kidney E2 (82408))	Expr (/evansm/Kidney, mouse/Study 2, U74v2/WT kidney E2 (82409))	Expr (/evansm/ Kidney, mouse/Study 2, U74v2/ERbKO kidney vehicle (82410))	Expr (/evansm/ Kidney, mouse/Study 2, U74v2/ERbKO kidney vehicle (82411))	Expr (/evansm/ Kidney, mouse/Study 2, U74v2/ERbKO kidney E2 (82412))	
x		137525_at	AI098139	Mm.38027		0.44	9	4	13	6	9	10	3	3	7	8	3	4
x		167023_f_at	AV016819	Mm.2808	biglycan	0.44	15	3	6	5	5	5	2	1	4	6	3	3
x		101738_at	U25145	Mm.57061	lutelnizing hormone beta	0.43	1120	578	608	262	525	470	180	125	257	446	176	165
x		104477_at	AW047643	Mm.28940		0.43	23	15	18	7	18	14	4	4	10	15	5	6
x		93104_at	Z16410		B-cell translocation gene 1, anti-proliferative	0.42	27	14	16	7	22	16	7	4	13	21	8	7
x		162969_at	AW123298	Mm.41716	Edil3 EGF-like repeats and discordin I-like domains 3	0.41	55	34	41	22	48	42	8	4	25	36	11	11
x		165569_at	AI847273	Mm.22305		0.41	14	5	16	6	15	15	6	4	7	13	5	5
x		170896_at	AV066592	Mm.34232	immune associated nucleotide 4	0.40	9	3	14	5	10	6	4	2	7	9	4	4
x		103729_at	M36775	Mm.243	laminin, alpha 1	0.40	24	17	26	7	25	11	6	3	14	18	8	4
x		132403_at	AI788603	Mm.169241	similar to TSC1_RAT HAMARTIN (TUBEROUS SCLEROSIS 1 PROTEIN HOMOLOG)	0.40	54	19	47	19	35	27	14	12	23	23	12	8
x		97519_at	X13986	Mm.321	secreted phosphoprotein 1	0.39	336	178	212	84	255	198	76	46	164	198	77	59
x		98055_at	AW121500	Mm.34330	bladder cancer associated protein homolog (human)	0.39	33	14	19	9	25	16	5	4	9	19	5	6
x		163224_at	AI843147	Mm.24577	IGSF1 immunoglobulin superfamily, member 1	0.38	51	27	33	17	31	26	6	5	18	26	4	7
x		95559_at	AI838836	Mm.27768	RIKEN cDNA 6330403K07 gene	0.38	145	74	73	27	86	71	23	12	42	64	25	19
x		99057_at	M12379		thymus cell antigen 1, theta	0.35	131	57	61	25	100	83	18	17	38	71	21	19
x		133139_at	AW122295	Mm.41642	regulator of G-protein signaling 4	0.34	20	4	22	6	12	8	4	2	6	8	4	4
x		162964_at	AI854153	Mm.41642	regulator of G-protein signaling 4	0.33	50	11	39	19	35	41	12	8	22	28	9	8
x		97520_s_at	X83569	Mm.140956	neuronatin	0.32	523	157	314	133	381	280	87	37	210	322	102	101
x		94694_at	M69196	Mm.1333	proprotein convertase subtilisin/kexin type 1	0.29	99	27	51	19	62	52	11	9	24	47	12	12
x		108851_at	AW125899	Mm.66275	Ras-like protein	0.27	33	10	33	6	24	28	8	6	38	14	8	10
x		101737_at	U12932	Mm.46711	follicle stimulating hormone beta	0.16	163	19	87	17	107	34	17	3	34	58	10	5

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